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ESTIMATING PATHOGEN RISKS AND CONCENTRATIONS IN SEWAGE SLUDGE FROM DISEASE INCIDENCE DATA IN AUSTRALIA AND MEXICO

Report to Smart Water Fund, Victoria, Australia

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Abstract

Biosolids reuse to land is both sustainable and an economically feasible alternative to landfill disposal or incineration. Stringent microbiological standards for biosolids in Victoria, Australia are barriers to reuse and include *Ascaris* ova removal requirements for unrestricted land application. This pathogen is largely absent from the local population making it difficult for water utilities to demonstrate these removals.

The effect of incidence of enteric disease on potential risk to human health from the agricultural use of conventionally-treated biosolids was investigated in the different socioeconomic contexts of Australia and Mexico. Data for prevalence of helminth infection in Australia is lacking. To assess risk when data is scarce, a model was developed to follow the partitioning and decay of nine pathogens from the infected population through to annual exposure from ingestion of uncooked root crops grown in biosolids amended soil. Using dose-response relationship data, annual risk of infection was estimated.

The model was validated using reported concentrations of pathogens in wastewater and sewage sludge and was found to be compliant for bacteria, protozoan (oo)cysts and helminths. Risk analysis revealed that *Ascaris* would incur a 10^{-1}pppy risk of infection in regions of high disease prevalence. Sensitivity analysis suggested that in regions where ascariasis incidence is low, risk of infection through biosolids reuse is also low.

The results of this investigation suggest that in regions such as Victoria, Australia, where ascariasis incidence is low and biosolids end use is controlled, a multi-barrier approach provides a safe and sustainable strategy to protect human health when biosolids are recycled to agricultural land. Enhanced treatment is required to minimise the risk of ascariasis from land application of biosolids in regions such as Mexico, where disease incidence is high and end use cannot be controlled.

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List of Abbreviations

ABS	Australian Bureau of Statistics
ADAS	Agricultural Development and Advisory Service
BPEO	Best practicable environmental option
BRC	British Retail Consortium
CDC	Centre for Disease Control
CEC	Council of European Communities
CONAGUA	Comisión Nacional del Agua, Mexico; National Water Comission (Mexico)
Defra	Department of food and rural affairs
DF	Distrito Federal; Federal District
DHA	Department of Health and Ageing (Australia)
DoE	Department of Environment
DS	dry solids
ECD	European Council directive
EHEC	Enterohaemorragic E. coli O157:H7
EPAV	Environmental Protection Agency, Victoria (Australia)
GDP	Gross National Product
HAV	Hepatitis A virus
HMSO	Her Majesty's Stationery Office
HO	helminth ova
ICD	International Classification of Diseases
INEGI	Instituto Nacional de Estadistica y Geografia; National Institute of Statistics and Geography (Mexico)
LEDC	less economically-developed country
MAD	Mesophilic anaerobic digestion
MEDC	more economically-developed country
N	dose or exposure
N_{50}	median infective dose
NWC	National Water Commission (Australia)
NWI	National Water Initiative (Australia)
OM	organic matter
p	probability of infection
p	probability of infection, or adverse response

PFRP	Processes to further reduce pathogens
pppy	per person per year
PSD	Pollution Solutions and Designs
PSRP	Processes to significantly reduce pathogens
QMRA	quantitative microbial risk assessment
r	infection success rate
RA	risk assessment
SEMARNAT	Secretaria del Medio Ambiente y Recursos Naturales; Ministry of Environment and Natural Resources (Mexico)
SSM	Safe Sludge Matrix
SUIVE	Sistema Único de Información para la Vigilancia Epidemiológica; Single Information System for Epidemiological Surveillance (Mexico)
tDS	tonnes of dry solids
USEPA	United States Environmental Protection Agency
UWWTD	Urban waste water treatment directive
WATSAN	Water and sanitation
WHO	World Health Organization
WSAA	Water Services Association of Australia
WWTP	wastewater treatment plant

1. Introduction

1.1. The agricultural reuse of sewage sludge

Sewage sludge is an inevitable by-product of wastewater treatment. Sludge production is increasing internationally due to increasingly stringent wastewater treatment standards, such as those imposed by the European Council Directive (ECD) 91/271/EEC (CEC, 1991), as well as more extensive sewerage connections (Defra, 2007). In England and Wales, sludge production was around 900 thousand tonnes of dry solids (tDS) per annum in 1986 and this increased to over 1.5 million tDS by 2005 (Defra, 2006; Defra, 2007; UN-HABITAT, 2008). In the USA, 6.5 million tDS of sludge were generated in 2004. Sludge production in Europe is expected to reach 9 million tDS in 2010 (UN-HABITAT, 2008). Recent figures for Mexican sludge production are unavailable, but recent efforts to promote its beneficial reuse (UN-HABITAT, 2008) suggest it is being generated at a substantial rate in that country.

In Australia, the state of Victoria produced 82,300 tDS in 2009, contributing to 23% of the sludge production in Australia and New Zealand that year (Spears, 2010). As can be seen in Figure 1.1, a large proportion of sludge has been stockpiled.

Ocean disposal of sewage sludge, once one of the main disposal methods used in England and Wales, was banned in Europe from the end of 1998 by ECD 91/271/EEC The Ocean Dumping Ban Act of 1988 banned sludge disposal at sea in the United States (Copeland, 1999).

Other sewage sludge disposal routes include: landfill, stockpiling and incineration. Stockpiling and landfilling of sludge are not considered as sustainable options for sludge management in the long term.

Sewage sludge, which contains a considerable organic content (12%: PSD, 2009) and significant liquid content (80%: PSD, 2009), is unsuitable for landfill disposal due to the increasing pressures against the landfilling of biodegradable and largely liquid wastes as they encourage the production of landfill gas and leachate (CEC, 1999).

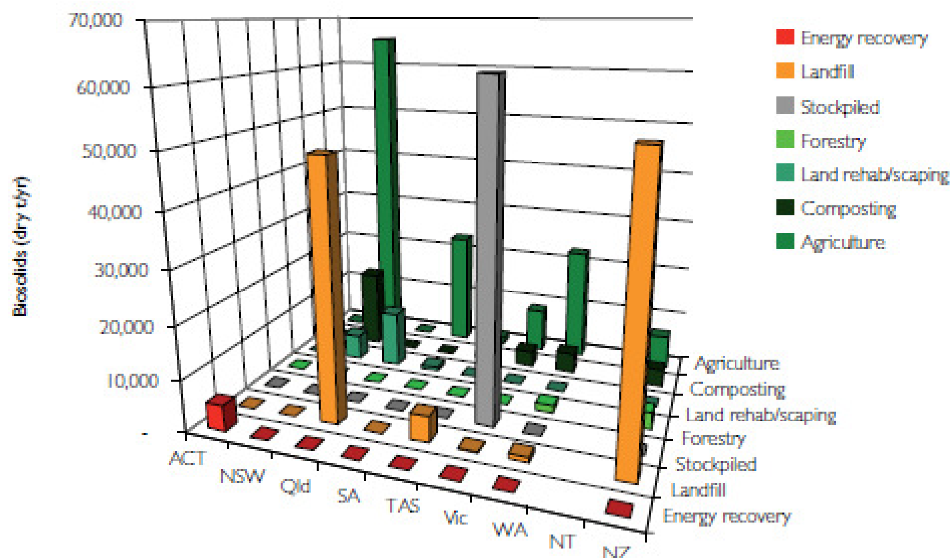


Figure 1.1. Annual production and uses of biosolids in Australia (state/territory) and New Zealand. (UN-HABITAT, 2008)

Incineration produces biologically sterile ash residues (Williams, 2005) therefore allowing them to be disposed in landfill or otherwise. This method also has the advantage of reducing the volume of the solid waste by approximately 90% (Williams, 2005). Large-scale incineration offers the possibility of energy neutrality but it requires considerable capital expenditure, is subject to regulations regarding gas emissions (Andrews *et al*., 1998) and is often opposed by the general public.

Sludge treated to a standard acceptable for land application is described as “biosolids”. Agricultural reuse of biosolids is a sustainable alternative to disposal as it takes advantage of the substance’s nutrient and organic matter (OM) content in sludge, which have significant agronomic value. However, it is critical that the land application route is demonstrably regulated and controlled to protect human health and the environment.

The increasing use of biosolids in agriculture in England and Wales, as well as the impact of ECDs 91/271/EEC and 1999/31/EC, is shown in Figure 1.2. In comparing sludge production in 1980 and in 2005, more stringent wastewater treatment required by ECD 91/271/EEC led to a >20% increase in the total amount of sludge produced. Disposal to landfill decreased in accordance with EDC 99/31/EEC, the Landfill Directive. The amount of biosolids being reused in agriculture nearly doubled.

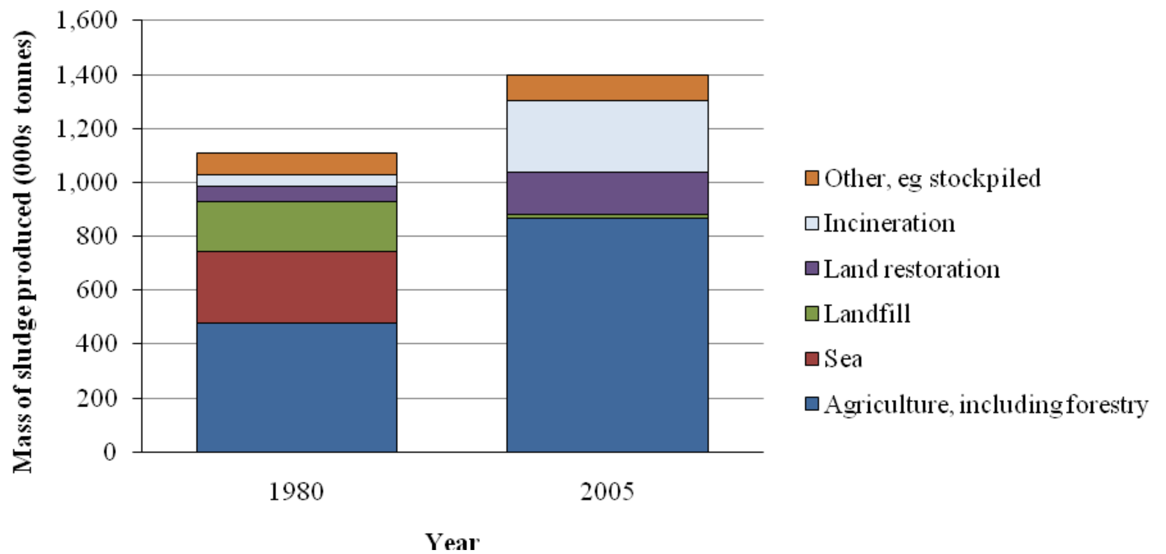


Figure 1.2. Sludge production and disposal in England and Wales, 1980 and 2005 (compiled from Andrews *et al*., 1998 and WaterUK, 2006)

Biosolids reuse alternatives include: crop fertilisation for food and non-food crops; forestry; public parks, golf courses and cemeteries; soil production and disturbed land rehabilitation (Bengtsson and Tillman, 2004; Pepper *et al.,* 2006). The reuse of biosolids in agriculture has been regarded as the best practicable environmental option (BPEO) by the UK government in most circumstances as it "aligns with the UWWTD and with the principals of the waste hierarchy of reduction, re-use, recovery and then disposal" (Defra, 2007).

Biosolids can be a suitable nutrient source for plants as it contains organic matter, nitrogen, phosphorus, potassium and trace elements such as calcium and magnesium (Wang, 1997; Pepper *et al*., 2008). The nutrient content of sludge is shown in Table 1.1. The agricultural reuse of biosolids offers several advantages over synthetic fertilisers: crop yields equal to or better than those offered by synthetic fertilisers (Wang, 1997), improvement of physical properties (Pepper *et al*., 2008); avoidance of eutrophication and the cost of nutrient-destructing processes (Jimenez *et al*., 2002); increased soil organic matter which otherwise tends to decrease with intensive agricultural practices (Wang, 1997); and the build-up of macronutrients such as carbon, nitrogen and phosphorus (Pepper *et al*., 2008) making them available for plant uptake rather than losing them to sinks such as water ecosystems. This is a matter of rising importance,

particularly with regards to vital nutrients such as phosphorus (Steen, 1998; Bengtsson and Tillman, 2004; Smith, 2007).

The man–sludge–soil–crop–man infection pathway is a potential mechanism of disease transmission from the agricultural reuse of biosolids (Jimenez *et al*., 2002) and it was therefore the focus of this investigation, but there are also further associated complications to be aware of: direct and indirect contamination of surface water through leaching (Dumontet *et al*., 2001), bioaerosol emissions (Pepper *et al*., 2008), and heavy metal and organic chemical contamination. However, Zerzghi (as cited by Pepper *et al*., 2008) showed that the risks associated with heavy metals were low, not hazardous, and decreased by source control measures over a 20-year period. Smith (2009) and Clarke and Smith (in press) also argue that impacts of organic contaminants in biosolids-amended soil are minimal.

Table 1.1. Reported nutrient content in digested biosolids. Values are expressed as % DS (dry solids)

	Australia	**UK**	**China**	**USA**
Total N	5.2	5.0	2.53	4.2
P	--	3.3	1.05	3.0
K	--	0.2	0.74	0.3
Reference	Rigby *et al.* (2010)	Goulding (2008)	Wang (1997)	Sommers (1977)

N – Nitrogen
P – Phosphorus
K – Potassium
-- not reported

1.2. The risk to human health from pathogens in biosolids

Risk of human enteric disease arises when a pathway is established from a pathogenic source to a human receptor. Sewage sludge contains a range of infectious enteric pathogens capable of causing infection in humans unless appropriate management in the form of sludge treatment is practised. Therefore, control measures are required to prevent the development of faecal-oral transmission pathways between sewage sludge pathogens and crop consumers. (Sterritt and Lester, 1988)

Understanding pathogen transmission barriers and survival rates in wastewater and sewage sludge treatments is necessary to evaluate the risk to human health posed by the land application of biosolids. Risk assessment provides a holistic analysis of the

available microbiological information to reach robust conclusions about the public health consequences of pathogen exposure. (Haas *et al.* 1999).

Opinion is largely that the risks to human health posed by biosolids reuse to agriculture are low (Strauch, *et al.*, 1991; Gale, 2002; Gerba *et al.*, 2002; Westrell *et al.*, 2004). No cases of disease outbreak have been reported following implementation of guidelines and codes of practice (Sludge (Use in Agriculture) Regulations 1989) to control pathogens in sludge. For example, in its 19th revision the UK Royal Commission on Environmental Pollution Sustainable Use of Biosolids noted that "there are no instances in the UK in which a link has been established between the controlled application of sewage sludge and occurrence of disease in the general population through food or water contamination" (HMSO, 1996; p.85).

Bruce *et al.* (1990) identified the following factors affecting the risk of disease transmission from the land application of biosolids:

- Prevalence of disease in the community
- Conditions of treatment of sludge
- Use and management of biosolids
- Management of animal rearing
- Pressures on the use of land receiving biosolids

This investigation focused on the first of these factors.

1.3. The relationship between enteric disease prevalence and socioeconomic development

Blumenthal *et al.* (2001) noted that, in order to provide a robust basis to legislative decisions, results from any given study must take into account the constraints of conditions relevant to a particular time and location. If an enteric disease is prevalent in a population, then this will be reflected in the pathogenic content of the resulting wastewater (Bates *et al.*, 1984; Bruce *et al.,* 1990).

As proposed by Sidhu and Toze (2009), pathogen variations in sewage sludge may result from different socioeconomic conditions. In a comparison with the work of Stoll (1947), Chan (1997) pointed out that widespread infection of intestinal helminths had disappeared from more economically-developed countries (MEDCs), but was still present in Latin America and the Caribbean, Sub-Saharan Africa and Asia.

When making the case for the reuse of wastewater in agriculture, Blumenthal *et al*. (2001) argued that stringent treatment standards can in fact be an unnecessary expense in MEDCs. Here, the incidence of enteric disease is already low and the pathogen input into the treatment process is therefore dealt with at source.

Biosolids classification guidelines in Victoria, Australia prescribe stringent microbiological standards for land application including helminths *Ascaris* and *Taenia* removals which water utilities have difficulty demonstrating due to low prevalence of these parasites in the community.

With the purpose of assessing the relevance of stringent microbiological controls over agricultural reuse of biosolids in Victoria, enteric disease incidence data from Victoria and Mexico have been studied to compare and contrast risks from pathogens in biosolids in different socioeconomic contexts.

2. Aims and objectives

2.1. Aims

International information on the incidence of human enteric disease will be collected and used to estimate the potential numbers of pathogens present in biosolids, and in turn, the presence of viable pathogens found in crops grown in sludge-amended soil. Risks to human health from the agricultural reuse of biosolids will be estimated.

To provide a basis for recommending appropriate microbiological quality standards for the agricultural reuse of biosolids in Victoria, Australia as well as Distrito Federal and Chiapas, Mexico.

2.2. Objectives

The objectives of the project are specifically to:

A. Identify the most significant enteric pathogens that can be transmitted through the wastewater and sludge cycles in Victoria and Mexico.

B. Determine the concentrations of pathogens entering a conventional wastewater treatment plant using disease incidence information in Victoria and Mexico.

C. Determine the change in numbers of significant pathogens through conventional wastewater and conventional sewage sludge treatment processes.

D. Estimate the potential number of pathogens present in sewage sludge for agricultural reuse in Victoria and Mexico following a standard treatment process.

E. Estimate the number of pathogens in contact with root crops grown in sludge-amended soil.

F. Use expected decay rates for pathogens in soil to estimate soil concentration of pathogens and crop contamination rates after prescribed waiting periods.

G. Estimate the related risks to human health from enteric pathogens in sludge recycling to farmland in Victoria and Mexico.

3. Literature review of theoretical and regulatory context

3.1. Single and multiple barriers

Faecal-oral transmission occurs when there is contact between the mouth and excreta containing pathogens. Contact may take place directly, from one host to another, or indirectly. Indirect contact may occur through contaminated food, drink, skin or vector organisms such as flies. Once ingested, the pathogen can then make its way through the host's alimentary canal into faeces (Sterritt and Lester, 1988). Faeces from infected humans are therefore a source of infectious agents in biosolids. For this reason, the number and types of pathogens in biosolids are influenced by the prevalence of disease in the community.

There are four main groups of infectious agents of potential concern for human health from agricultural reuse of sewage sludge and they include: viruses, bacteria, protozoa and helminths (Straub *et al*., 1993)

Microbiological risks to humans coming from the agricultural use of biosolids are minimised by applying single or multiple barriers to break transmission pathways. For instance, treatment reduces the risk of disease transmission by inactivating pathogens and reducing the vector-attraction potential of biosolids. Barriers governed by biosolids reuse regulations include: sludge treatment, crop restrictions and time intervals before harvesting or grazing on biosolids-amended soil (Godfree and Farrell, 2005).

Single barriers are designed to allow use of biosolids in outlets where land use not controlled, such as domestic gardening. Pathogen elimination, or reduction to background levels, is achieved through what is described in the UK as "enhanced" sludge treatment processes. In this case land use measures are not required to protect human health and sludge treated to this level may be used without restriction. In the UK, these high microbiological quality biosolids treated are described as "enhanced treated". In the USA, biosolids of similar microbiological standard are described as "Class A".

The multiple barrier approach is designed for situations, such as general agricultural use, where land use can be managed and there is no technical justification to treat sludge to enhanced status incurring the high costs and energy consumption usually required when treating sludge to this standard. This approach consists of several barriers that act additively to prevent pathogen transmission from raw wastewater in to land-applied biosolids. The multi-barrier approach requires sludge treatment to significantly reduce though not necessarily to eliminate pathogens, followed by land use restrictions to allow natural attenuation of pathogens in soil. In the UK, biosolids of this microbiological quality are described as being "conventionally" treated. The equivalent term in the USA is "Class B".

For example, under the Safe Sludge Matrix (SSM) in the UK, salad and vegetable crops, which are likely to be consumed raw, may only be grown on land amended with enhanced-treated biosolids or an extended harvest interval must be applied (ADAS, 2001). Following land application, further inactivation occurs in the soil environment. The length of pre-established time intervals between biosolids application and harvesting and/or grazing allows sufficient further inactivation to take place. The SSM is shown in Table 3.1.

Table 3.1. The Safe Sludge Matrix (ADAS, 2001)

CROP GROUP	UNTREATED SLUDGES	CONVENTIONALLY TREATED SLUDGES	ENHANCED TREATED SLUDGES
FRUIT	✗	✗	✓ (10 month harvest interval applies)
SALADS	✗	✗ (30 month harvest interval applies)	✓ (10 month harvest interval applies)
VEGETABLES	✗	✗ (12 month harvest interval applies)	✓ (10 month harvest interval applies)
HORTICULTURE	✗	✗	✓ (10 month harvest interval applies)
COMBINABLE & ANIMAL FEED CROPS	✗	✓	✓
GRASS & FORAGE - GRAZED	✗	✗ (Deep injected or ploughed down only) (3 week no grazing and harvest interval applies)	✓ (3 week no grazing and harvest interval applies)
GRASS & FORAGE - HARVESTED	✗	✓ (No grazing in season of application) (3 week no grazing and harvest interval applies)	✓ (3 week no grazing and harvest interval applies)

NOTE: ✓ All applications must comply with the Sludge (Use in Agriculture) Regulations and DETR Code of Practice for Agricultural Use of Sewage Sludge.

✗ Applications not allowed (except where stated conditions apply)

3.2. Regulatory context

Legislation on biosolids reuse to agricultural land is generally comprised of management of two types of risks to human health and the environment: chemical and microbiological. The current investigation focused on the microbiological aspects of biosolids management. Therefore, regulations relating to biosolids pathogen content in the UK, USA, Victoria, Australia; and Mexico are outlined in this chapter.

Microbiological standards are used to classify biosolids into different grades for reuse, thus allowing the highest microbiological-quality biosolids to be used with limited or no restriction. The underlying rationale is that stringent treatment ensures a single barrier to disease transmission by achieving a very high inactivation of all pathogens of concern to human health. A multiple-barrier approach is used to regulate the reuse of biosolids of lesser microbiological quality through the implementation of end-use restrictions such as the types of crops grown and harvest periods. A summary of some international biosolids classifications can be found in Table 3.2.

3.2.1. United Kingdom

Biosolids reuse in the UK is governed by the Sludge (Use in Agriculture) Regulations 1989 and additional measures stipulated in the Safe Sludge Matrix (Table 3.1) (ADAS, 2001). Enhanced-treated biosolids may be used without restrictions on land use as they have undergone a 6 log reduction in *E. coli*, contain less than 10^3 *E. coli* g^{-1}DS (per gram dry solids) and do not contain *Salmonella*. In conventional biosolids treatment, for instance mesophilic anaerobic digestion (MAD), pathogen concentrations are significantly reduced, but not necessarily eliminated and therefore restrictions on land application are required. The microbiological quality guidelines for conventionally-treated biosolids require a 2 log reduction in faecal coliforms (FC) or < 10^5 MPN g^{-1}DS. Land use restrictions that apply to both categories of biosolids are listed in the Safe Sludge Matrix (Table 3.1).

3.2.2. United States

Agriculturally-reusable biosolids in the United States are classified according to microbiological quality as dictated by the Environmental Protection Agency *Part 503 Regulations for the Use or Disposal of Sewage Sludge* (CFR, 1995).

Table 3.2. Pathogen quality classification systems for biosolids in different countries

Country	Sludge classification	Faecal coliforms	*Salmonella*	Enteric viruses	*Ascaris* ova	*Taenia* ova
UK [a]	**Enhanced**	$<10^3$ g^{-1}DS OR >6 log removal	zero (2g^{-1}DS)			
	Conventional	$<10^5$ g^{-1}DS OR >2 log removal				
USA [b]	**Class A**	$<10^3$ g^{-1}DS	<3 (4g^{-1}DS)	<1 PFU (4g^{-1} DS)	<1 (4g^{-1}DS)	
	Class B	$<2x10^6$ g^{-1}DS				
Victoria, Australia [c]	**T1**	$<10^2$ g^{-1}DS	<1 (50g^{-1}DS)	<1 (100 g^{-1} DS) OR >3 log removal	>2 log removal	
	T2	$<10^3$ g^{-1}DS	<10 (50g^{-1}DS)	<2 (100 g^{-1} DS) OR >2 log removal		<1 (10g^{-1}DS) OR >2 log removal
	T3	$<2x10^6$ g^{-1}DS	>1 log removal	>1 log removal		
Mexico [d]	**Clase A**	$<10^3$ g^{-1}DS	<3 g^{-1}DS		<1 g^{-1}DS	
	Clase B	$<10^3$ g^{-1}DS	<3 g^{-1}DS		<10 g^{-1}DS	
	Clase C	$<2x10^6$ g^{-1}DS	<300 g^{-1}DS		<35 g^{-1}DS	

[a] Sludge (Use in Agriculture) Regulations 1989; ADAS (2001)
[b] CFR (1995)
[c] EPAV (2004)
[d] SEMARNAT (2003)

Microbiological guidelines for Class A biosolids include < 10^3 FC g^{-1}DS as well as detection-limit thresholds for *Salmonella*, enteric viruses and helminth ova (Table 3.2), which are achieved through treatment by Processes to Further Reduce Pathogens (PFRP). Biosolids meeting these guidelines may be used without site restrictions, including residential purposes or on lawns. Class B biosolids are contain < $2x10^6$ FC g^{-1}DS and therefore their land application requires restrictions on crop harvesting, animal grazing and public access. Biosolids treatment processes that achieve Class B microbiological standards are described as "Processes to Significantly Reduce Pathogens" (PSRP) and include MAD (USEPA, 2003)

Treatment processes and/or conditions for pathogen reduction other than those prescribed by the USEPA (2003) may demonstrate compliance with required densities of FC or *Salmonella*, as well as enteric viruses and helminth ova. However, the presence of enteric viruses and helminth ova in wastewater and sewage sludge is dictated by the disease prevalence in the community, and may be absent from raw sludge altogether thus complicating the demonstration of the required removals for these pathogens.

The difficulty of demonstrating the required enteric virus and helminth ova removals in areas of low disease prevalence can be overcome by presuming Class A biosolids are being produced while ongoing monitoring of enteric virus and viable helminth ova shows these are not present in untreated sludge. As long as operation conditions are maintained, monitoring may continue until these pathogens are detected at which point the treatment efficiency will be able to be tested. As microbiological data can take several weeks to be processed, USEPA recommends water utilities keep biosolids on-site until sampling and analyses are completed.

With regards to Class B biosolids, PSRP equivalence can be established if bacterial and viral reductions of at least 1 log are achieved. Otherwise, 2 log FC removal is accepted as a robust parallel indicator or, alternatively, FC population of fewer than 10^6 g^{-1}DS. These removals are reportedly obtained by MAD at 35°C over 15 days and therefore any processes that do not deviate significantly from conventional sludge treatment[1] are accepted as Class B equivalents provided faecal coliform and faecal streptococci counts do not exceed 10^6 g^{-1}DS.

[1] Thickening, anaerobic/aerobic biological treatment, dewatering, air drying and storage of liquid sludge or sludge cake.

3.2.3. Victoria

In Victoria, biosolids reuse is regulated by the Environmental Protection Agency *Guidelines for Environmental Management: Biosolids Land Application* (EPAV, 2004). Once a contaminant grade has been assigned to the biosolids, a treatment grade is determined by use of a prescribed treatment process stated in the guidelines. If the treatment process is not listed as a prescribed process, verification or intensive batch testing must take place to demonstrate that it achieves the required pathogen removals (Table 3.2).

The highest microbiological-quality biosolids, fit for unrestricted use, are classified as T1. Provided their contaminant grade is also high, T1 biosolids that qualify for unrestricted use can be used in residential contexts as well as on food crops that grow near the ground surface and are likely to be consumed raw. End-use restrictions of the land-spreading of lesser microbiological-quality T2 and T3 biosolids include: control of public access, types of crops grown, waiting periods between biosolids application and harvest (Table 3.3). In Victoria, MAD is recognised as a T3-grade biosolids treatment process.

To be verified as T1-grade, alternative treatments must demonstrate, in addition to the *Salmonella* removals and *E. coli* removals required for prescribed treatment processes, > 3 log removal of enteric viruses and > 2 log removal of *Ascaris* ova. The > 2 log removal of *Ascaris* ova required for verification of a T1-grade process equivalent is a barrier to classifying biosolids suitable for reuse. Due to the low prevalence of ascariasis in Australia, water utilities wishing to validate alternative T1 treatment-grade processes cannot demonstrate the required removal.

To be validated as T2-compliant, processes must demonstrate >2 log reductions in enteric virus, and *Taenia* ova, in addition to *Salmonella* and *E coli* requirements shown in Table 3.2. Validation of alternative T3-grade processes requires enteric virus and *Salmonella* removals of >1 log and $< 2\text{x}10^6$ FC g^{-1}DS. In Victoria, MAD is recognised as a T3-grade biosolids treatment process.

Biosolids reuse practice, and even policy, may be made more precautionary than is technically justified by lack of confidence and understanding of microbiological risks;

for instance, widespread use of Class A biosolids for Class B biosolids applications in the US state of California (Pepper *et al.*, 2006). In general, the agricultural application of sludge treated to such a high standard of microbiological quality is unjustified in public health terms and may also be unsustainable due to the high energy demand and cost (Mara and Horan, 2002).

Table 3.3. Biosolids classification and permitted end use in Victoria, Australia (EPAV, 2004, p.34)

			"Restricted uses"					
			Agricultural uses			Non-agricultural uses		
Treatment grade	Chemical grade	**"Unrestricted"** [a]	Human food crops consumed raw in direct contact with biosolids [b]	Dairy and cattle grazing/ fodder (also poultry), human food crops consumed raw but not in direct contact [c]	Processed food crops [d]	Sheep grazing and fodder (also horses, goats), on food crops, woodlots [e]	Landscaping (unrestricted public access) [f]	Landscaping (restricted public access), forestry, land rehabilitation [g]
T1	C1	√	√	√	√	√	√	√
T2	C1	x	x	√	√	√	√	√
T3	C1	x	x	x	√	√	x	√
T1	C2	x	√	√	√	√	√	√
T2	C2	x	x	√	√	√	√	√
T3	C2	x	x	x	√	√	x	√

√ The biosolids grade will generally be acceptable for the end use. Biosolids less than T1C1 will be subject to management controls.
x Biosolids of this quality are not acceptable for the end use (would require a risk assessment and site specific EPA approval/ licensing)
a Unrestricted uses – biosolids are suitable for distribution, marketing and appropriate use with only minimal controls, includes sale as a bagged product for residential use.
b Human food crops potentially consumed raw and in direct contact with biosolids.
c Human food crops potentially consumed raw but not in direct contact with biosolids includes those grown on trees.
d Processed food crops refer to crops that are either cooked at greater than 70°C for two minutes or processed (such as cereals, wheat and grapes for wine production) prior to sale to the domestic market.
e Non-human food crops include turf, woodlots, flowers and ornamental plants.
f Landscaping with unrestricted public access – includes public parks and sports grounds, with controls on access during soil renovation and until fully vegetated.
g Landscaping with restricted public access, land restoration – includes non-recreational land, road development, rehabilitation of quarries, mines and landfills, sewage treatment plants and other landscaping where there is controlled or limited public and stock access. Forestry also involves restricted public access and stock access.

In Victoria, T3 microbiological quality is equivalent to conventional (UK) or Class B (USA) but there appears to be reluctance amongst operators to developing the general agricultural application of T3 biosolids and the focus is on achieving T1 grade removals For instance, the preferred process stream in Victoria is MAD followed by air-drying and long-term storage for three years. Consequently, this has contributed to low

recycling rates in Victoria and the more than 2 million tonnes of long-term stockpiling of biosolids in the state.

3.2.4. Mexico

USEPA Rule 503 is the basis for biosolids reuse legislation in Mexico NOM-004-SEMARNAT-2002 (SEMARNAT, 2003). Biosolids are microbiologically classified as A, B or C, depending on their FC, *Salmonella* and helminth ova content as shown in Table 3.2.

Reuse of Class C biosolids is permitted for agricultural, land rehabilitation and forestry. Class B biosolids may also be used for in urban situations as long as direct contact with the public is avoided during application. In addition to Class B and C uses, Class A biosolids are considered fit for urban use including direct contact during application.

The development of wastewater infrastructure in Mexico, as with other middle-income countries, has largely focussed on provision of sanitary services and wastewater treatment (particularly in urban areas). However, interest in harvesting biosolids as a resource, and therefore, in sludge treatment and management has grown (UN-HABITAT, 2008).

3.3. Quantitative microbial risk assessment

Risk assessment provides a scientific basis for the safe agricultural reuse of sewage sludge. Quantitative microbial risk assessment (QMRA) was developed to assess the potential risks to health from the spread of infectious disease by assessing the impact of exposure to infectious pathogens. (Haas *et al*. 1999). QMRA can be used to determine the effectiveness of treatment and management factors by minimising the risk.
The key steps in developing a QMRA include (Haas *et al.,* 1999; Gerba *et al*. 2008):

(a) **Hazard identification.** An understanding of microbial hazards as causes of disease is developed including diagnostic tools, the disease process, transmission routes, microorganism life cycles and approaches to treatment.

(b) **Exposure assessment.** The amount of microorganisms constituting a "dose", or single exposure, is established.

(c) **Dose-response assessment.** The relationship between different levels of exposure to pathogens and the probability of an adverse consequence is established.

(d) **Risk characterisation.** Exposure and dose-response assessments are brought together into a "risk statement". The risk statement is usually a quantitative measure of outcome which is appropriate to the purposes of a particular QMRA. For instance, expected risk of infection to a person and expected disease prevalence in a community are examples of risk statements.

3.4. Published QMRA for biosolids-amended soil

Using information about pathogen concentrations and removal rates, Gale (2002; 2003) designed pathogen-specific microbiological event trees for conventional wastewater treatment, sludge treatment and sludge management dictated by the Safe Sludge Matrix, SSM (ADAS, 2001). After a series of partitions, the concentration of a given pathogen was converted into an estimated pathogen density on a crop.

The event tree procedure was used to assess the risk to human health from consumption of salad and root crops grown in biosolids-amended soil in accordance with the SSM. Reported as estimated number of potential infections in the population, the assessment was performed for a variety of enteric pathogens in enhanced and conventionally-treated biosolids. Harvest periods stipulated in the SSM for conventionally-treated biosolids were technically justified as they reduced risk from *Salmonella, Listeria monocytogenes, E. coli O157, Campylobacter, Giardia* and enteroviruses to less than one infection in the UK per ten million years. In the case of *Cryptosporidium*, the risk was one infection every 45 years (Gale, 2002).

Mara and Horan (2002) determined the annual risk of *Salmonella* infection from potatoes grown in soil amended with conventionally-treated biosolids was $2\text{x}10^{-4}$ per person per year (pppy); for lettuce, this value was $2\ \text{x}10^{-5}$ pppy. They concluded, therefore, that the risk associated with growing crops for human consumption on land

on which conventionally-treated biosolids had been applied was very low. In the case of lettuce, this was lower than the risk for drinking water accepted by the USEPA: 10^{-4} pppy from a single exposure (Regli *et al.,* 1991).

Gerba *et al.* (2002) employed the QMRA approach developed by Haas *et al.* (1999) to assess the survival of emerging pathogens adenovirus and hepatitis A virus (HAV) through selected sludge treatment processes. If exposure to these pathogens took place through ingestion of treated biosolids, risk of infection rose above the 10^{-4}pppy limit allowed by USEPA, and was therefore unacceptable. Risks from viruses were reduced to an acceptable level if biosolids were diluted in soil at the time of application, through ploughing or subsurface injection, for example.

Westrell *et al*. (2004) assessed the risks of enteric pathogen exposure associated with biosolids reuse in Sweden to prioritize the pathogenic hazards to be controlled. Risk of exposure was set within different scenarios – ranging from bioaerosol inhalation to ingestion of raw vegetables grown in biosolids-amended soil – on four components: (1) volume ingested; (2) frequency of exposure; (3) number of affected individuals and (4) infectivity of the pathogen in question. The community impact of exposure through the various scenarios was assessed by comparing it with pre-existing disease prevalence and considering disease severity. A worst-case harvest time of one month after sludge application was used in the RA rather than the ten months indicated by Swedish legislation. The impact of crops grown in biosolids-amended soil was found to be less than four other exposure scenarios, all of which involved direct contact with sludge: WWTP workers at pre-aeration and belt-press, child playing at sludge storage and entrepreneur spreading sludge. It was concluded that the impact of consumption of raw vegetables grown in biosolids-amended soil was low for *Salmonella, Giardia, Cryptosporidium*, rotavirus and adenovirus; although a major impact could result from enterohaemorragic *E.coli* O157:H7 (EHEC) infection due to the disease severity based on the conservative assumptions adopted in the model. The results are shown in Table 3.4. Helminth infections were not included in the investigation, presumably because helminths do not present a microbiological problem in Sweden.

Table. 3.4. **The impact of infection of enteric disease resulting from consumption of raw vegetables grown in biosolids-amended soil in Sweden.** (Westrell *et al*., 2004)

Pathogen	Median	95% confidence interval

	Annual infections resulting from exposure	Impact of infection [a]	Annual infections resulting from exposure	Impact of infection [a]
EHEC [b]	0.002	Insignificant	0-0.01	Major
Salmonella	0	Insignificant	0-0.003	Insignificant
Giardia	0.002	Insignificant	0-0.025	Insignificant
Cryptosporidium	0.01	Minor	0.0004-0.15	Moderate
Rotavirus	0.21	Insignificant	0.01-2.91	Minor
Adenovirus	0.41	Minor	0.01-20.51	Moderate

[a] Defined by the authors depending on the magnitude of the annual infection value and disease severity
[b] Enterohaemorragic *E. coli* O157:H7

Pepper *et al.* (2006) estimated the risk of rotavirus infection for a child ingesting Class B biosolids as 8.6 x10^{-4}pppy. If biosolids were diluted by soil application the risk decreased to 1.8x10^{-6}pppy. Waiting periods between application and public access were not taken into account in either case. The results were below the maximum risk of rotavirus infection allowed by the USEPA (Regli *et al.,* 1991).

Gerba *et al.* (2008) compared the risk associated with exposure to Class B biosolids to that of Class A biosolids in which *Salmonella* regrowth had occurred after treatment. Biosolids *Salmonella* concentration and exposure were identified as significant variables dictating risk of infection. Within the context of the precautionary assumptions of the investigation, the risk associated with Class B biosolids was found to be close to the 10^{-4} upper limit suggested by USEPA, whereas risk from direct contact with Class A biosolids could be higher than that if regrowth occurred.

Navarro *et al*. (2009) determined an *Ascaris* dose-response relationship based on Mexican epidemiological data. QMRA was performed to assess the risk of land application of biosolids containing *Ascaris* ova concentrations ranging from 0.25 to 37 ova g^{-1}DS. The study concluded that raising the helminth ova threshold for biosolids destined for land application did not significantly increase risk of infection. The authors suggested that, in a LEDC context, unaffordable costs of achieving excessively stringent helminth ova limits could be minimised by implementing evidence-based standards and multiple barriers such as land use restrictions and improved washing practices.

3.5. Dose-response assessment

3.5.1. Purpose of dose-response assessment

The aim of dose-response assessment is to establish the relationship between level of exposure to pathogens (or "dose") and the probability of an adverse reaction, such as infection, occurring as a result (or "response"). The dose-response relationship for an epidemiological study can be fitted mathematically and therefore extrapolated to assess risks of infection associated with low doses. (Haas *et al.,* 1999)

3.5.2. Dose-response models

Exponential

The simplest dose-response model is the negative exponential described by Equation (3.1) where N represents the exposure, or dose, and p stands for the probability of an adverse reaction (in this case, infection). A graphical example of the exponential dose-response relationship is shown in Figure 3.1.

$$p = 1 - e^{-rN} \tag{3.1}$$

Parameter r is the "infection success rate", the probability that a pathogenic organism will survive long enough to initiate an infection. The assumption that, for a given pathogen species, organisms are equally independent and have equivalent survival probabilities results in this model's constant r value (Haas *et al.*, 1999; Gale, 2001).

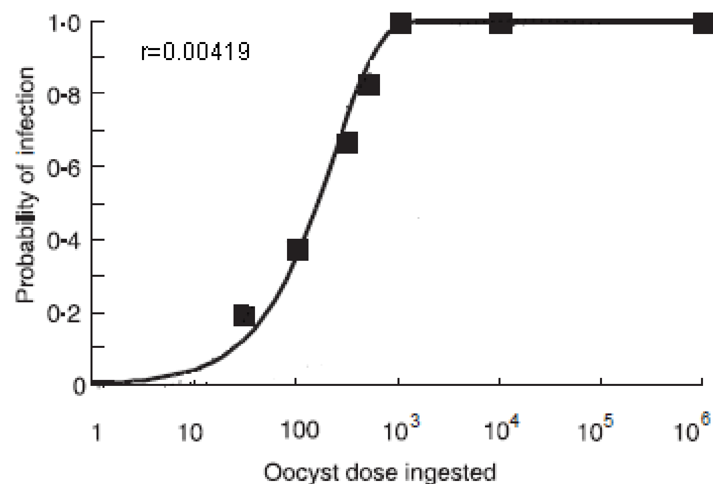


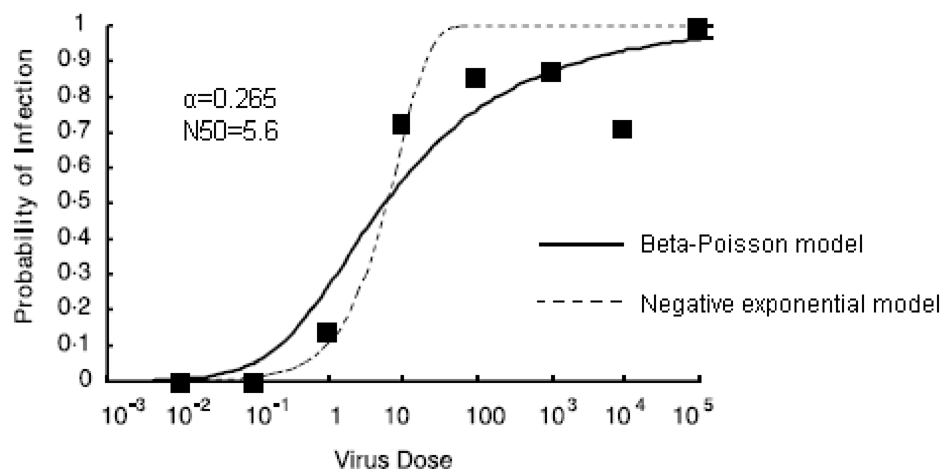
Figure 3.1. Exponential dose-response curve for *Cryptosporidium parvum* oocysts. (Haas *et al.*, 1996).

Beta-Poisson dose-response relationship

In some cases parameter *r* is not constant for reasons including variation of microorganism infectivity. Often *r* is beta-distributed, leading to the Beta-Poisson dose-response relationship described by Equation (3.2) and represented graphically in Figure 3.2.

$$p = 1 - \left(1 + \frac{N}{\beta}\right)^{-\alpha} \tag{3.2}$$

Parameters α and β are shape parameters of a beta-distribution density function (Haas *et al.*, 1999; Fazil, 2005).



F igure 3.2. **Beta-Poisson dose-response curve for rotavirus.** Negative exponential model shown for comparison (Gale, 2001; Haas *et al.*, 1993; Ward *et al.*, 1986).

Often it is useful to express probability of infection in terms of mean infectious dose, N_{50}, which is given by Equation 3.3.

$$N_{50} = \frac{\ln(0.5)}{-r} \tag{3.3}$$

The Beta-Poisson relationship given by Equation 3.2 can be rearranged to express probability of infection terms of N_{50} (Haas *et al.*, 1999):

$$p = 1 - \left[1 + \frac{N}{N_{50}}\left(2^{1/\alpha} - 1\right)\right]^{-\alpha} \quad (3.4)$$

Table 3.5. Reported dose-response relationships for a selection of pathogens

Pathogen	Dose-response relationship	Parameters	Reference
Viruses			
Rotavirus	(3.4)	$\alpha = 0.26; N_{50} = 5.6$	Haas *et al.* (1993)
Bacteria			
Campylobacter	(3.2)	$\alpha = 0.15; \beta = 7.9$	Teunis *et al.* (1999)
Salmonella	(3.2)	$\alpha = 0.3136; \beta = 3{,}008$	FAO/WHO (2000)
	(3.2)	$\alpha = 0.89; \beta = 440{,}000$	Teunis *et al.* (1999)
	(3.2)	$\alpha = 0.7; \beta = 3.5 \times 10^{9}$	Coleman and Marks (2000)
***E. coli* O157:H7**	(3.4)	$\alpha = 0.16; N_{50} = 1130$	Crockett *et al.* (1996)
	(3.2)	$\alpha = 0.221; \beta = 8722.46$	Powell *et al.* (2000)
Protozoan (oo)cysts			
Cryptosporidium	(3.1)	$r = 0.00035$	Okhuysen *et al.* (1998)
	(3.1)	$r = 0.000091$	Chapell *et al.* (1999)
Giardia	(3.1)	$r = 0.0198$	Rose *et al.* (1991)
Helminth ova			
Ascaris	(3.4)	$\alpha = 0.104; N_{50} = 859$	Navarro *et al.* (2009)

4. Literature review of data used to construct and validate the QMRA model developed

4.1. Excretion of enteric pathogens

Pathogen density in the faeces of an infected individual were reported by Feachem (1983). The values given for selected bacteria, protozoa and helminth ova agree with those reported in more recent investigations, shown in Table 4.1. The uncertainty of Hepatitis A virus (HAV) and rotavirus concentration values of $10^6 g^{-1}$ faeces was noted by Feachem and are several orders of magnitude greater in more recent literature (Ward *et al*., 1986; Straub *et al*., 1993; Schwartzbrod *et al*., 1995; Gerba *et al*., 1996; Pepper, *et al*., 2006) which may be due to enhanced virus enumeration methods.

Virus, *Giardia* cyst and *Campylobacter* concentrations in faeces have been well documented, while data for *Taenia* ova is scarce.

The mean range of pathogen density in faeces is flanked by virus and helminth ova excretion, at $10^{11} g^{-1}$ and $10^{-4}\ g^{-1}$ faeces, respectively. Bacteria and protozoa concentrations lie within the two extremes. The range reported by Taylor *et al.* (1993) is unusually broad, perhaps because the immunity of subjects of the study, children under 5 years of age, was highly variable.

Table 4.1. Reported pathogen densities in faeces of an infected individual

	Mean density (g^{-1} faeces)	Reported density (g^{-1} faeces)	
Viruses			
Hepatitis A virus	$5.5x10^{11}$		
		$1x10^{6}$	Feachem *et al.* (1983)
		$1x10^{12}$	Ward *et al.* (1986)
		$1x10^{11}$	Pepper *et al*. (2006)
Rotavirus	$3.0x10^{11}$		
		$1x10^{6}$	Feachem *et al.* (1983)
		$1x10^{12}$	Ward *et al.* (1986)
		$1x10^{10}$	Straub *et al*. (1993); Gerba *et al.* (1996)
		$1x10^{11}$	Schwartzbrod *et al*.(1995); Pepper *et al*. (2006)
Bacteria			
Campylobacter	$2.2x10^{8}$		
		$1x10^{6}–1x10^{9}$	Blaser *et al.* (1979) as cited by Taylor *et al.* (1993)
		$1x10^{7}$	Feachem *et al.* (1983)
		$1x10^{1}–1x10^{8}$	Taylor *et al.* (1993)
Salmonella	$5.1x10^{7}$		
		$1x10^{8}$	Feachem *et al.* (1983)
		$1x10^{6}$	Toze (1997)
Shigella	$2.8x10^{7}$		
		$1x10^{5}–1x10^{8}$	Dale and Mata (1968)
		$1x10^{6}$	Toze (1997)

Table 4.1 *continued*. Reported pathogen densities in faeces of an infected individual

	Mean density (g^{-1} faeces)	Reported density (g^{-1} faeces)	
Protozoan (oo)cysts			
***Cryptosporidium* oocysts**	2.5×10^7		
		2.5×10^7	Medema *et al.* (2001)
***Giardia* cysts**	3.2×10^6		
		1×10^7	Danzinger and Lopez (1975) as cited by Horan and Mara (2003)
		1×10^5	Feachem (1983)
		1×10^6	Jakubowski (1984); Medema *et al.* (2001)
Helminth ova			
***Ascaris* ova**	1×10^4		
		1×10^4	Feachem *et al.*(1983); Toze (1997)
***Taenia* ova**	1×10^4		
		1×10^4	Feachem *et al.* (1983)

4.2. Pathogens in wastewater treatment

4.2.1. Pathogen concentration in raw wastewater

Pathogen and indicator concentrations in raw wastewater have been widely reported, particularly rotavirus, *Salmonella*, *Cryptosporidium* oocysts, *Giardia* cysts and *Ascaris* ova (Table 4.2) although the latter have mostly been reported in LEDCs. Data for *Shigella* and *Taenia* ova are scarce.

In spite of the broad ranges reported, bacteria were present in wastewater in the highest concentrations (reaching 10^9 MPN L^{-1}, most probable number per litre, in Mexico; Jimenez, *et al.* 2001) and appear to be present at higher densities in LEDC wastewater. Virus and protozoan (oo)cyst concentrations fall within the range of 10^2 to 10^4 MPN L^{-1}. Exceptions are the unusually low 10^{-1} MPN L^{-1} *Cryptosporidium* oocyst lower limit reported by Yates and Gerba (1998) and unusually high 10^7 plaque-forming unit per litre (PFU L^{-1}) HAV concentration found by Albinana-Gimenez *et al.* (2006, as cited by Sidhu and Toze, 2009). The latter may be have been due to varying sensitivity of virus enumeration methods (Sidhu and Toze, 2009).

4.2.2. Pathogen transfers into primary sludge

In primary sedimentation, a portion of wastewater suspended solids are transferred to the resulting primary sludge. Some wastewater pathogens remain in the effluent and continue into secondary treatment. Others, particularly those sorbed onto solids (as some viruses) or are relatively large (such as helminth ova) will settle into the primary sludge (Straub *et al.*, 2003; Jimenez *et al.*, 1998). Removals of helminth ova from wastewater are indicated by the large partitioning for *Ascaris* and *Taenia* ova reported by Newton *et al.* (1984), EPA (1992; as cited by Jimenez *et al.*, 1998) and Cooper and Olivieri (1998).

These and other reported transfers of pathogens from wastewater into primary sludge are shown in Table 4.3. Of the pathogens reported, viruses displayed the greatest range: from zero to 70% (Feachem, 1980, as cited by Lucero-Ramirez, 2000; Cooper and Olivieri, 1998). Such variations may result from differences in detection and extraction efficiency.

Table 4.2. Pathogen concentrations in raw wastewater

Pathogen	Concentration in raw wastewater (L^{-1}) Mean	Range	Location	Reference
Viruses [a]				
Hepatitis A virus [a]	1.4×10^7	n/a		Albinana-Gimenez *et al.*, (2006) as cited by Sidhu and Toze (2009)
Rotavirus [a]	3.1×10^4	$5.1 \times 10^3 – 9.6 \times 10^4$	Brazil	Oragui *et al.* (1989) as cited by Gerba *et al.* (1996)
	1.4×10^4	n/a		Bosch *et al.* (1988) as cited by Sidhu and Toze (2009)
	5.4×10^3	$1 \times 10^3 – 1.4 \times 10^4$	Spain	Bosch *et al.* (1988) as cited by Gerba (1996)
	4.4×10^2	$1.4 \times 10^1 – 3.0 \times 10^3$	USA	Rao *et al.* (1986)
	1.56×10^2	$2.5 \times 10^1 – 6.5 \times 10^2$	Spain	Bosch *et al.* (1988) as cited by Gerba *et al.* (1996)
	10^2	n/a		Loddeer *et al.* (2005) as cited by Sidhu and Toze (2009)
		$4 \times 10^2 – 8.5 \times 10^4$		Yates and Gerba (1998)
Enteric virus [a]	3×10^4	n/a		Cooper and Olivieri (1998)
Bacteria [b]				
Campylobacter [b]	5.53×10^4	n/a	UK	Arimi *et al.* (1988)
Salmonella [b]	4×10^3	n/a		Cooper and Olivieri (1998)
	3.3×10^3	n/a	UK	Yaziz and Lloyd (1979)
	n/a	$7.8 \times 10^8 – 6.5 \times 10^9$	Mexico	Jimenez *et al.* (2001)
	n/a	$4.5 \times 10^6 – 2.4 \times 10^6$	Mexico	Jimenez *et al.* (2001)
Shigella [b]	$10^1 – 10^4$	n/a		Yates and Gerba (1998)
Protozoan (oo)cysts				
***Cryptosporidium* oocysts**	4.8×10^3	$8.3 \times 10^2 – 1.3 \times 10^4$	Canada	Chauret *et al.* (1999)
	2×10^2	n/a		Cooper and Olivieri (1998)
	4.9×10^2	n/a		Robertson *et al.* (2000)
	n/a	$3 \times 10^{-1} – 4 \times 10^3$		Yates and Gerba (1998)

Table 4.2 *continued*. Pathogen concentrations in raw wastewater

Pathogen	Concentration in raw wastewater (L^{-1}) Mean	Range	Location	Reference
***Giardia* cysts**	1.6×10^4	n/a		Robertson *et al* (2000)
	7.6×10^2	n/a	Tunisia	Ayed *et al*. (2009)
	4.8×10^3	$5.1 \times 10^2 – 1.8 \times 10^4$	Morocco	Amahmid *et al*. (1999)
	n/a	$1.25 \times 10^2 – 2 \times 10^5$		Yates and Gerba (2008)
	2×10^2	n/a		Cooper and Olivieri (1998)
	n/a	$1 \times 10^2 – 2 \times 10^4$	Norway	Robertson *et al.* (2006)
	n/a	$3 \times 10^2 – 8.9 \times 10^3$	Norway	Robertson *et al.* (2006)
	8.3×10^1	$1 \times 10^3 – 2 \times 10^4$	Canada	Chauret *et al*. (1999)
Helminth ova				
Total helminth ova	8×10^2	n/a		Cooper and Olivieri (1998)
***Ascaris* ova**	1.7×10^2	$1.3 \times 10^1 – 6.7 \times 10^2$	Pakistan	Ensink *et al.* (2007)
	n/a	$2.4 \times 10^1 – 2.7 \times 10^1$	Mexico	Jimenez *et al*. (2001)
	n/a	$5 \times 10^0 – 1.1 \times 10^2$		Yates and Gerba (2008)
	n/a	$6.6 \times 10^0 – 1.5 \times 10^1$	Mexico	Navarro *et al*. (2009)
	4.6×10^2	n/a	Tunisia	Ayed *et al*. (2009)
	3.9×10^1	$7 \times 10^{-1} – 1.3 \times 10^1$	Morocco	Amahmid *et al*. (1999)
***Taenia* ova**	5.1×10^1	n/a	Tunisia	Ayed *et al*. (2009)

n/a – not applicable or unavailable

[a] PFU – plaque forming unit

[b] MPN – most probable number

Table 4.3. Pathogen transfers into primary sludge

Pathogen	Percentage transfer into sludge (%)	Comment	Reference
Viruses[a]	5 – 10		Payment *et al*. (2001)
Enterovirus	11		Irving and Smith (1981) as cited by Gale (2002)
Enteric virus	70		Cooper and Olivieri (1998)
	0 – 30		Feachem (1980) as cited by Lucero-Ramirez (2000)
Bacteriophage	49.9	Argentina, Colombia, France, Spain	Lucena *et al*. (2004)
Bacteria	50 – 90		Feachem (1980) as cited by Lucero-Ramirez (2000)
Faecal coliforms [b]	65.3		Chauret *et al*. (1999)
	60.2	Argentina, France, Spain	Lucena *et al*. (2004)
Campylobacter	63.8		Arimi *et al*. (1988)
	78		Arimi *et al*. (1988)
Salmonella	81		Yaziz and Lloyd (1979)
	50		Cooper and Olivieri (1998)
Protozoan (oo)cysts	10 – 50		Feachem (1980) as cited by Lucero-Ramirez (2000)
***Cryptosporidium* oocysts**	92.9 (49.9 – 0.99)		Chauret *et al*. (1999)
	18.6		Robertson *et al* (2000)
***Giardia* cysts**	22.4		Chauret *et al.* (1999)
	37.7		Robertson *et al.* (2000)
	50		Cooper and Olivieri (1998)
Helminth ova			
Total helminths	90		Cooper and Olivieri (1998)
***Ascaris* ova**	90		EPA (1992) as cited by Jimenez *et al*. (1998)
***Taenia* ova**	98	2hrs sedimentation	Newton *et al.* (1948)

[a] Enterovirus, enteric virus and bacteriophage were used as indicators of rotavirus and HAV behaviour

[b] Faecal coliform were used as indicator of *Shigella* behaviour.

4.2.3. Pathogen inactivation in the activated sludge process

For many pathogens in primary effluent some degree of inactivation takes place in secondary biological treatment. Decay of viruses, bacteria and protozoan oocysts and cysts during activated sludge process (ASP) is well documented (Table 4.4). From these data, it appears removals were highest for bacteria (mean removal 93%), followed by viruses (mean removal 90%) and finally protozoan cysts and oocysts (mean removal 88%), although faecal coliform removals have been reported over a broad range of 0.79log (Dehab and Surampalli, 2002) to 3.52log (Chauret *et al.*, 1991).

4.2.4. Pathogen transfer into secondary sludge

Secondary sludge results from the sedimentation of suspended solids in effluent from ASP. As with primary sedimentation, a portion of pathogens settle into the sludge, while some continue in the resulting effluent.

Transfers into secondary sludge of widely-studied pathogens *Campylobacter*, *Salmonella*, *Cryptosporidium* oocysts, *Giardia* cysts and *Ascaris* ova, as well as a generic “virus” category are shown in Table 4.5.

Table 4.4. Pathogen removals during the activated sludge process (ASP)

Pathogen	Percentage reduction (%)	Comment	Reference
Viruses[a]			
Enterovirus	80 – 99		Rao *et al.* (1986)
Bacteriophage	93.7	Argentina, France, Spain	Lucena *et al.* (2004)
Bacteria [b]			
E.coli	87.4		Gantzer *et al.* (2001)
Faecal coliforms	99.97		Chauret *et al.* (1999)
	83.8		Dehab and Surampalli (2002)
	99 – 99.9	Czech Republic	Zabranska *et al*. (2003)
	92.1	Argentina, France, Spain	Lucena *et al.* (2004)
Campylobacter	98		Koenraad *et al.* (1997)
Salmonella	90		Geldreich *et al.* (1972)
Protozoan (oo)cysts			
***Cryptosporidium* oocysts**	90		Rose and Canahan (1992) as cited by Pepper (2006)
	99.89		Chauret *et al.* (1999)
	93	Laboratory	Villacorta-Martinez *et al.* (1992)
	75		Medema and Schiven (2001)
***Giardia* cysts**	90		Rose and Canahan (1992) as cited by Pepper (2006)
	94.9		Chauret *et al.* (1999)
	77.6		Payment *et al*. (2001)
	80		Gale (2002)
	90		Medema and Schiven (2001)

[a] Enterovirus and bacteriophage were used as indicators of rotavirus and HAV behaviour

[b] *E coli* and faecal coliforms were used as indicators of *Shigella* behaviour

Table 4.5. Pathogen transfers into secondary sludge

Pathogen	Percentage transfer into sludge (%)	Comment	Reference
Viruses	90		Rao *et al*. (1986); Bitton (1999)
Bacteria			
Campylobacter	90	Reading WWTP	Arimi *et al.* (1988)
Salmonella	90		Gale (2002)
Protozoan (oo)cysts			
***Cryptosporidium* oocysts**	90		Gale (2002)
***Giardia* cysts**	90		Gale (2002)
Helminth ova			
***Ascaris* ova**	99.7	Laboratory	Wen *et al*. (2009)

4.2.5. Pathogen concentration in treated wastewater

Table 4.6 shows pathogen and indicator concentrations in effluent following primary sedimentation and biological ASP treatment. Viruses were present at the largest concentrations, reporting up to 10^3 PFU L^{-1} (Bates, 1984; Bosch, 1988, as cited by Gerba *et al.,* 1996).

Data for *Cryptosporidium* oocysts in Canada (Chauret *et al*., 1999) and Tunisia (Ayed *et al*., 2009) suggest pathogen concentration differences relating to socioeconomic development.

4.3. Pathogens in sewage sludge treatment

4.3.1. Pathogen concentrations in raw sludge

Throughout wastewater treatment, pathogens become concentrated in raw sludge, which consists of a mixture of primary and secondary sludge. The presence of pathogens and indicators in raw sludge has been widely reported in a variety of countries, particularly *Salmonella*, *Giardia* cysts and *Ascaris* (Table 4.7). Viruses, *Shigella* and *Taenia* ova have not received as much attention.

Bacteria have been present at the greatest concentrations, but also within the greatest range: 10^1 (Horan *et al*. 2004) to 10^8 MPN L^{-1} (Jimenez *et al*, 2002, as cited by Dumontet *et al.*, 2001), or even 10^{11} MPN L^{-1} if a model is included (Gale, 2002).

Giardia cyst concentration data have been reported for a variety of countries and suggest higher densities in LEDCs Mexico and Tunisia (Jimenez *et al.*, 2002; Ayed *et al.,*2009) than in MEDCs France, Canada, and USA (Thriat *et al.*, 1997, as cited by Jimenez *et al.*, 2002; Chauret *et al.,* 1999; Jimenez *et al.,* 2002), although the concentrations reported for France are slightly higher than for other MEDCs.

Helminth ova concentrations as high as 10^2 HO g^{-1}DS (helminth ova per gram of dry solids) have been reported in Mexico on three occasions (Jimenez *et al.,* 2002; Nelson, 2003). Concentrations in Brazil and MEDCs France, UK and USA lower by at least one

order of magnitude (Thomaz-Soccol *et al.,* 1997; Crewe *et al.*, 1997, as cited by Jimenez *et al.* (2002); Gaspard *et al.*, 1997).

Table 4.6. Pathogen concentrations in effluent following primary and secondary treatment

Pathogen	Concentration in effluent (L^{-1}) Mean	Range	Location	Reference
Viruses [a]				
Rotavirus [a]	$7.5x10^2$	$0 -1.5x10^3$	UK	Bates *et al*. (1984)
	$1.0x10^3$	$4.8x10^1 – 3.2x10^3$	USA	Bosch *et al*.(1988) as cited by Gerba *et al.* (1996)
Bacteria [b]				
Campylobacter [b]	$4.0x10^1$	n/a	UK	Arimi *et al*. (1988)
Protozoan (oo)cysts				
***Cryptosporidium* oocysts**	$5.1x10^1$	$<1.25x10^0 – 1.7x10^2$	Canada	Chauret *et al*. (1999)
***Giardia* cysts**	$3.3x10^0$	$1.9x10^{-1} – 7.5x10^0$	Canada	Chauret *et al*. (1999)
	$2.0x10^2$	n/a		Casson *et al.* (1990) as cited by Gale (2002)[2]
		$2.2x10^2 – 9.6x10^2$	Tunisia	Ayed *et al*. (2009)
Helminth ova				
***Ascaris* ova**	$4.6x10^1$	n/a	Tunisia	Ayed *et al*. (2009)
***Taenia* ova**	$6x10^0$	n/a	Tunisia	Ayed *et al*. (2009)

n/a – not applicable or unavailable

[a] PFU – plaque forming unit

[b] MPN – most probable number

[2] Based on predicted removal

Table 4.7. Pathogen concentrations in raw mixed primary and secondary sewage sludge before MAD

Pathogen		Concentration in raw sludge (g DS^{-1})		Location	Reference
		Mean	Range		
Viruses [a]		$1.6x10^{5c}$	$2.5x10^{3c} – 8.0x10^{5c}$	USA	Berg and Berman (1980)
	Enteric virus [a]	$1x10^{3}$	n/a		Cooper and Olivieri (1998)
Bacteria [b]					
	Campylobacter	$1.3x10^{11}$	n/a	Model	Gale (2002)
		10^{5c}	n/a		Jones *et al*. (2001)
	Salmonella [b]	$2x10^{3}$	n/a		Cooper and Olivieri (1998)
		n/a	$10^{6} – 10^{8}$	Mexico	Jimenez *et al*. (2002)
		$2.3x10^{3}$	n/a	USA	Pedersen (1981) as cited by Jimenez (2002)
		n/a	$2.0x10^{1} – 4.0x10^{1}$	Sweden	Horan *et al*. (2004)
		n/a	$10^{3} – 10^{6}$		Dumontet *et al*. (2001)
		$2.9x10^{2}$	n/a		Pederson (1981)
		$1.01x10^{7}$	n/a	Model	Gale (2002)
		10^{3}	n/a	USA	Jimenez *et al*. (2002)
		$1.1x10^{4}$	$1x10^{2} – 7.0x10^{4}$	USA	Gale (2001)
	Shigella [b]	10^{7}	n/a		DeBertoldi *et al.* (1983) as cited by Dumontet *et al.* (2001)
Protozoan (oo)cysts					
	***Cryptosporidium* oocysts**	$1.4x10^{1}$	$1.2 x10^{1} – 1.7 x10^{1}$	Poland	Graczyk *et al*. (2008)
		10^{2}	n/a	Model	Gale (2002)
		$5.3 x10^{0}$	$<2.5x10^{-1} – 3.8 x10^{1}$	Canada	Chauret *et al*. (1999)

Table 4.7 *continued.* Pathogen concentrations in raw mixed primary and secondary sewage sludge before MAD

Pathogen	Concentration in raw sludge (g DS^{-1})		Location	Reference
	Mean	**Range**		
***Giardia* cysts**	$1x10^2$	n/a		Cooper and Olivieri (1998)
	n/a	$1.3x10^2 – 4.2x10^4$	Mexico	Pedersen (1981) as cited by Jimenez *et al*. (2002)
	n/a	$10^2 – 10^4$		Jimenez *et al*. (2002)
	n/a	$1.2 x10^2 – 6.5 x10^2$	Tunisia	Ayed *et al*. (2009)
	n/a	$2.9x10^4$	Model	Gale (2002)
	$1x10^2$	n/a	USA	Jimenez *et al*. (2002)
	$2.7 x10^1$	$2.2 x10^1 – 3.2 x10^1$	Poland	Graczyk *et al*. (2008)
	$4.4x10^0$	$<2.5x10^{-1} – 1.2 x10^1$	Canada	Chauret *et al*. (1999)
	n/a	$1.9x10^3 – 5.7x10^3$	France	Thriat *et al*. (1997) as cited by Jimenez *et al*. (2002)
Helminth ova	$3x10^1$	n/a		Cooper and Olivieri (1998)
	n/a	$7.3 x10^1 – 1.8 x10^2$	Mexico	Jimenez *et al*. (2002)
	$4.9 x10^0$	n/a	Brazil	Thomaz-Soccol *et al.* (1997)
	n/a	$4.9 x10^1 – 6.6 x10^2$	Mexico	Nelson *et al*. (2003)
	n/a	$2.5 x10^1 – 2.6 x10^2$	Mexico[3]	Nelson *et al*. (2003)
	n/a	$<2.5x10^{-1} – 7 x10^0$	France	Banas *et al.* (2003) as cited by Sidhu and Toze (2009)
	n/a	$2.4 x10^0 – 9.0 x10^0$	UK	Crewe *et al*. (1997) as cited by Jimenez *et al*. (2002)
	n/a	$6 x10^{-1} – 2.4x10^0$	France	Gaspard *et al*. (1997)
	n/a	$<1 – 10^1$	USA[4]	Jimenez *et al*. (2002)

n/a – not applicable or unavailable

[a] PFU – plaque forming unit

[b] MPN – most probable number

[c] Values originally reported as L^{-1} wet sludge and converted to g^{-1} DS using same assumptions as those used to construct this investigation's model.

[3] Viable ova

[4] Viable ova

4.3.2. Pathogen inactivation achieved by mesophilic anaerobic digestion

Mesophilic anaerobic digestion (MAD) is widely practised and considered to be a standard conventional treatment method by the regulators mentioned in Chapter 3 (USEPA, 1993; ADAS, 2001; EPAV, 2004). Pathogen inactivation during MAD has therefore been studied extensively, especially for faecal coliforms which require a 2 log reduction by conventional treatment under the Safe Sludge Matrix, SSM. The mean of the removals of faecal coliforms and *E. coli* shown in Table 4.8. are 2.35 log and 2.16 log respectively, and therefore consistent with SSM requirements for the standard practice of digestion at 35°C for 15 days followed by 14 day-storage.

Ascaris ova are generally resistant to MAD and destruction requires enhanced treatment or long-term storage and/or drying. They are not, however always present in sludge and are particularly scarce in MEDCs, which explains why a large proportion of *Ascaris* data reported is from LEDCs (numerous examples can be found in Tables 4.2, 4.6 and 4.7).

Two significantly different *Giardia* cyst removals were reported: 3 log and no reduction by Gavaghan *et al*. (1993) and Chauret *et al.* (1999), respectively.

Table 4.8. Pathogen removals during mesophilic anaerobic digestion (MAD)

Pathogen		Percentage reduction (%)	Comment	Reference
Viruses		89 (76 – 96)	20d; 35°C	Berg and Berman (1980)
	Rotavirus	51.0 – 99.99994		Spillman *et al.* (1987)
	Enterovirus	93.8 (91.1 – 95.6)		Pederson (1981)
		90		Gerba *et al*. (2002)
	Bacteriophage	98.4		Lasobras, *et al*. (1999)
Bacteria				
	E.coli	99.3 (95.5 – 99.96)	Laboratory	Horan *et al*. (2004)
		96.8		Gantzer *et al*. (2001)
		99 – 99.9		Zábranská *et al*. (2003)
		92.1 – 98.0	Laboratory	Lang and Smith (2008)
	Faecal coliforms	98.9		Dahab and Surampalli (2002)
		96.1		De Leon *et al*. (2002) as cited by Sidhu and Toze (2009)
		99		Watanabe (1997)
		99		Lafitte-Trouque (2002)
		99.7	35°C, 50d	Lucero-Ramirez (2000)
	Campylobacter	56.3	Laboratory	Horan *et al*. (2004)
		99.9		Jones *et al*. (1990)
		92		Jones *et al*. (1990)
		90		Kearney *et al*. (1993)

[a] Enterovirus and bacteriophage were used as indicators of HAV behaviour

[b] *E coli* and faecal coliforms were used as indicators of *Shigella* behaviour

Table 4.8 *continued*. Pathogen removals during mesophilic anaerobic digestion (MAD)

Pathogen	Percentage reduction (%)	Comment	Reference
Salmonella	98.0		Horan *et al*. (2004)
	99.993 (99.991 – 99.995)	Seftenberg, laboratory	Horan *et al*. (2004)
	86.2 – 99.5		Dahab and Surampalli (2002)
	96 – 98		Lang and Smith (2008)
	99.994	Seftenberg	Gale (2002)
	97.7 (87.7 – 99.2)		Pedersen (1981)
	99.1		Gerba *et al*. (2008)
	98.9	Laboratory, 35°, 45d	Lucero-Ramirez (2000)
Protozoan (oo)cysts			
***Cryptosporidium* oocysts**	49.9		Chauret *et al*. (1999)
	99.64		Horan and Lowe (2001)
***Giardia* cysts**	99.9		Gavaghan *et al.*(1993)
	0		Chauret *et al*.(1999)
Helminth ova			
***Ascaris* ova**	30 – 50		Bowman and Fayer (2005)
	0		Carrington *et al*. (1993)
	0		Ganzter *et al*. (2001)
***Taenia* ova**	99		Storey *et al.* (1987)
	99		Pike (1986)

4.3.3. Pathogen concentration in treated biosolids

Reported concentrations of several pathogens biosolids following mesophilic anaerobic digestion (MAD) are shown in Table. 4.9. This has been studied extensively. *Salmonella* and *Ascaris* ova have received much attention whereas virus data is lacking.

As in raw sludge, bacteria were the most abundant pathogens in treated biosolids but also present over a large range (10^{-1} – 10^{6} MPN g^{-1}DS).

4.4. Pathogen decay in soil

Reported decay rates for pathogens in soil are shown in Table 4.10.

Under the multi-barrier approach for biosolids management, prescribed waiting periods are implemented as a pathogen inactivation step after conventional treatment. Pathogen decay occurs in the period of time between biosolids application and crop harvesting to ensure pathogen inactivation has occurred before harvesting crops grown in biosolids-amended soil.

To aid comparison, and for later use in this investigation, the reported values were extrapolated to 10 months as this is the minimum harvest waiting period for following biosolids land application in the Safe Sludge Matrix (Table 3.1). This time interval applies to enhanced-treated biosolids-amended soil on which food crops are grown. (For conventionally-treated biosolids, the waiting period rises to 12 months and 30 months for vegetable crops and salad crops respectively.)

In extrapolating pathogen inactivation over the ten-month period, decay rates were assumed to be linear (Gale, 2002) however, recent work *Salmonella* spp. and *E. coli* were found to decay exponentially (Cass, 2009) and reach background levels sooner than if linear decay took place. Therefore *Salmonella* and *E. coli* decay rates shown here are in fact conservative.

Table 4.9. Pathogen concentrations in sewage sludge treated with mesophilic anaerobic digestion (MAD)

Pathogen	Concentration in treated sludge (gDS^{-1})		Location	Reference
	Mean	Range		
Viruses				
Rotavirus	n/a	14 – 485		Straub *et al*. (1993)
Bacteria				
Faecal coliforms [b]	$1.39x10^4$	n/a	Texas	Lucero-Ramirez (2000)
	n/a	$10^2 – 10^6$		Straub *et al*. (1993)
Campylobacter [b]	$1.3x10^2$	n/a	Model	Gale (2002)
Salmonella [b]	$5 x10^{-1}$	n/a	Texas	Lucero-Ramirez (2000)
	n/a	$10^1 – 10^2$		Strauch *et al*. (1991)
	n/a	$1 x10^0 – 4 x10^2$		Zaleski *et al*. (2005)
	$5.8 x10^{-1}$	n/a	Model	Gale (2002)
	n/a	$3 x10^0 – 10^3$		Straub *et al*. (1993)
	$1.1x10^2$	$1x10^0 – 4.8x10^2$	USA	Gale (2001)
Shigella	$2.0x10^1$	n/a		Straub *et al*. (1993)
Protozoan (oo)cysts				
***Cryptosporidium* oocysts**	10^0	n/a	Model	Gale (2002)
	10^2	n/a		Hu *et al*. (1996)
	2.65	$<2.5x10^{-1} – 5.4 x10^0$	Canada	Chauret *et al*. (1999)
***Giardia* cysts**	$1.3 x10^{-1}$	$<2.5x10^{-1} – 2.1 x10^1$	Canada	Chauret *et al*. (1999)
	n/a	$10^2 – 10^3$		Straub *et al*. (1993)
Helminth ova				
***Ascaris* ova**	n/a	$2.4x10^{-2} – 1x10^{-1}$	Czech Republic	Horak *et al.* (1992)
	$7x10^{-1}$	n/a	North USA	Bowman and Fayer (2005)
	$9.6x10^0$	n/a	SE USA	Bowman and Fayer (2005)
	n/a	$1.4 x10^0 – 9.7 x10^0$	USA	Pedersen (1981) as cited by Jimenez (2002)
	n/a	$6.6 x10^1 – 1.4 x10^2$	Mexico	Pedersen (1981) as cited by Jimenez (2002)

n/a – not applicable or unavailable   [a] PFU – plaque forming unit   [b] MPN – most probable number

Ascaris ova showed by far the lowest decay rates in Table 4.10, not even reaching 1 log over a 10-month period. They can persist in soil over several years; (Straub *et al.*, 1993; Smith *et al.*, 1999; Pepper *et al.*, 2006). Other pathogens exhibited rapid decays, such as the bacteria *Campylobacter* (45.5 log decay over 10 years). The range of HAV decay rates is noteworthy as it has been reported as 1.7 log to 32.2 log depending on soil type and temperature (Sobsey *et al.*, 1995).

Table 4.10. Pathogen decay in soil over a ten-month period

Pathogen	Log Reduction	Reference
Viruses[a]		
Hepatitis A virus	1.7 – 31.2	Sobsey *et al.* (1995)
Enterovirus	13.5	Tierney (1977)
Bacteriophage	25	Hurst *et al.* (1980)
Bacteria		
Campylobacter	45.5	Hutchinson, *et al*. (2002) as cited by Gale (2002)
Salmonella	18.3	Gale (2002)
***E.coli* O157:H7**[b]	6.5	Gale (2002)
Protozoan (oo)cysts		
***Cryptosporidium* oocysts**	3.6	Olson *et al.* (1999)
	3	Hutchinson *et al*. (2002) as cited by Gale (2002)
***Giardia* cysts**	10	Gale (2002)
Helminth ova		
Ascaris	0.33	Gaasenbeek and Borgsteede (1998)
Taenia	5 – 7	Storey *et al.* (1987)

[a] Enterovirus and bacteriophage were used as indicators of rotavirus behaviour
[b] *E. coli* O157:H7 was used as an indicator of *Shigella* behaviour

5. Methodology

5.1. Summary of modelling approach

The QMRA method proposed by Gale (2002) has been applied to assess the risks of treated biosolids reuse in agriculture. The wastewater and sludge treatment processes are mapped as a series of partitions (known as "event trees") which can be used to estimate pathogen numbers. These operate under the assumption that multiple barriers to pathogen transmission act additively: so decay in soil removes any viable pathogens still present in biosolids after treatment. This assumption is supported by the work of Watson (1980) and Hu *et al.* (1996).

Gale (2002) developed two event trees: one to show the pathogen partitions taking place in wastewater treatment and another to show pathogen partitions in sludge treatment and application onto soil. Estimated pathogen loading on to a crop was then used to estimate the probability of infection from its uncooked consumption.

The novel approach developed here applies the event tree technique to predict the potential risk to human health when microbiological data are scarce based on infection rates in the population of notifiable enteric diseases. Event trees aid the transparency of the risk assessment procedure as they report information and methodologies clearly and concisely.

The QMRA was developed as follows:

(a) **Hazard identification.** The source of microbiological risk under consideration was the excretion of enteric pathogens by an infected population. Diseases caused by nine known enteric pathogens have been considered: two viruses, three bacteria, two protozoa and two helminths (Chapter 5.2). Incidence of disease in two countries was studied (Chapters 5.3).

(b) **Exposure assessment.** This phase was the emphasis of the investigation: to estimate annual exposure of an individual to faecal pathogens resulting from ingestion of raw vegetables grown in conventionally-treated (Chapter 5.4) biosolids-amended soil.

a. Pathogen excretion rates from infected individuals in the population were used to estimate the pathogen load in raw wastewater (Chapters 5.5 and 5.6).

b. Microbiological event trees were used present the pathogen flows through the treatment and end use pathways based on the work of Gale (2002). The first of these event trees used reported pathogen transfers and decay in conventional wastewater treatment to calculate the pathogen concentration in raw sewage sludge (Chapter 5.7).

c. Reported pathogen decays in conventional sludge treatment were used in a second event tree to estimate pathogen concentration in treated biosolids after prescribed harvest periods. Biosolids reuse information was used to estimate pathogen loadings at point of harvest of root crops likely to be eaten raw, such as carrots, and grown in biosolids-amended soil (Chapter 5.8).

d. Information on yearly vegetable consumption and biosolids use were used to estimate annual exposure to sewage sludge pathogens from consumption of crops grown in biosolids-amended soil (Chapter 5.9).

(c) **Dose-response assessment.** Reported dose-response relationships for various pathogens were compiled from the literature. These are detailed in Chapter 3.5.

(d) **Risk characterisation.** Finally, dose-response information and annual exposure to pathogens were integrated in Chapter 5.10 to estimate probability of infection and assess the risk to human health posed by given pathogen concentrations.

The resulting values for pathogen concentrations in raw and treated wastewater, as well as raw and treated sludge, were compared to the available literature to confirm the validity of the model. To account for variation in reported pathogen removals (Chapter 4), the QMRA was carried out twice: once using "typical" pathogen inactivation, and another using "worst-case" pathogen inactivation.

In some cases, notably ascariasis, taeniasis and cysticercosis, in which the diseases are not reported in Victoria (CDPCU, 2010), sensitivity analysis was employed by carrying out various iterations of hypothetical infections: starting with 1 infection and increasing

by one order of magnitude until the resulting risk of infection was comparable to that in Mexico's Distrito Federal (DF).

A worked example for *Giardia lamblia* is given in Chapter 5.11. Risk assessment for other pathogens can be found in Appendices B to J. Sensitivity analyses are provided in Appendices K and L.

5.2. Pathogens tested

5.2.1. Infectious agents

A variety of pathogens were tested including at least two from each of the major groups of infectious agents: viruses, bacteria, protozoa and helminths. The organisms and their respective diseases are shown in Table 5.1.

Table 5.1. Enteric pathogens tested and their associated diseases

Infectious agent	Species	Disease caused	ICD [a] code
Viruses			
	Hepatitis A virus	Infectious hepatitis	B15
	Rotavirus	Gastroenteritis	A08.0
Bacteria			
	Campylobacter spp.	Campylobacteriosis	A04.5
	Salmonella spp.	Salmonellosis, typhoid fever and paratyphoid fever	A01, A02
	Shigella spp.	Shigellosis	A03
Protozoa			
	Cryptosporidium spp.	Cryptosporidiosis	A07.2
	Giardia lamblia	Giardiasis (lambliasis)	A.07.1
Helminths			
	Ascaris	Ascariasis	B77
	Taenia spp.	Taeniasis and cysticercosis	B68, B69

[a] International Statistical Classification of Diseases and Related Health Problems 10th Revision (WHO, 2007)

Due to the significant difference in disease severity, typhoid fever is reported separately from other salmonellosis infections. However, the pathogens are the same species so their survival throughout the wastewater and sludge treatment trains, as well as infectivities, are similar. Hence, a distinction between paratyphi, typhi and other

*Salmonella*e species is rarely made. The disease incidence information for paratyphoid and typhoid fevers, as well as other salmonelloses, was compiled together to get a more complete overview of the occurrence of *Salmonella* spp. in wastewater and sludge.

Depending on the *Taenia* species causing an infection, the parasite ova may or may not require a secondary animal host to develop into infective cysticerci. *Taenia solium*, pork tapeworm, ova can develop into cysticerci in either pork or human muscle. *Taenia saginata* ova must go through an obligatory intermediate stage in cattle during which the larvae develop into cysticercii. Cysticercosis is the name given to the infection caused by ingestion of ova which then develops into a cysticercus Taeniasis develops when human ingestion of meat containing viable cysticerci takes place (CDC, 2009; WHO, 2010).

Incidence of both taeniasis and cysticercosis were combined to quantify the risks from *Taenia* spp. in this investigation. This offered the added advantage of ensuring non-zero pathogen concentrations for Distrito Federal (DF) as there had been no reported cases of *Taenia*sis in that state in 2008.

5.2.2. Use of surrogate indicator organisms

Removal data for pathogens such as HAV, rotavirus and *Shigella* was not always available. In these cases, data for indicator organisms was used instead. These replacements are summarized in Table 5.2.

Table 5.2. Use of surrogate indicator organisms. The organisms listed in the table were used to represent behaviour of HAV, rotavirus and *Shigella* at various points in the wastewater and sewage sludge treatment processes.

Process	HAV	Rotavirus	*Shigella*
Transfer to primary sludge	Enterovirus Enteric virus Bacteriophage	Enterovirus Enteric virus Bacteriophage	Faecal coliforms
ASP	Enterovirus Bacteriophage	Enterovirus Bacteriophage	*E. coli* Faecal coliforms
Transfer to secondary sludge	--	--	*Campylobacter* *Salmonella*
MAD	Enterovirus Bacteriophage	--	*E. coli* Faecal coliforms
Decay in soil	--	Enterovirus Bacteriophage	*E. coli O157:H7*
Dose-response relationship	Rotavirus	--	*E. coli O157:H7*

-- Use of indicator unnecessary, as data available for this organism

5.3. Country selection

The results of international investigations suggest low helminth ova concentrations occur in sludge of MEDCs than in LEDCs. For instance, Jimenez *et al.* (2000) reported 73 – 177 *Ascaris* ova g^{-1} DS in Mexican sludge; whereas Barbier, *et al.*, 1990 reported 0 – 9 helminth ova g^{-1} DS in sludge in France. These and further examples of LEDC and MEDC helminth concentrations in sludge are listed in Table 5.3. Gross National Product per capita (GNP per capita) has been included as an indicator of socioeconomic development.

Ascaris ova can only be removed from biosolids by enhanced treatment methods (Pepper *et al.*, 2006), which are not under consideration in this investigation. Because of their resistance to conventional treatment, variations in *Ascaris* ova densities in sludge are likely be due to variations in the pathogenic input into the system.

Table 5.3. Concentration of helminth ova in raw sludge in different countries

Country	2002 GNP per capita (USD) [a]	Concentration of helminth ova (HO g^{-1}DS) [b]	Reference
Mexico	6,230	66 – 136 [d]	Pedersen (1981) as cited by Jimenez *et al.* (2002)
		73 – 177 [d]	Jimenez *et al.* (2002)
Mexico (Mexico City)		49 – 657	Nelson (2003)
Mexico (Mexico City)		25 – 257 [d]	Nelson (2003)
France	24,770	<0.25 – 7	Schwarzbrod and Banas (2003)
		0 – 9	Barbier *et al.* (1990)
		0.6 – 8.9	Gaspard *et al.* (1997)
USA	37,610	<1 – 10 [d]	Jimenez *et al.* (2002)
		1.4 – 9.7 [c,d]	Pedersen (1981) as cited by Jimenez *et al.* (2002)
USA (North)		0.7 [c,d]	Reimers *et al.* (1986)
USA (South East)		9.6 [c,d]	Reimers *et al.* (1986)

[a] WHO (2007) [b] HO g^{-1} DS – helminth ova per gram of dry solids
[c] *Ascaris* lumbricoides ova only [d] viable helminth ova

By choosing to analyse data from Mexico, a comparison was drawn between the microbiological quality of sewage sludge resulting from a conventional treatment train in a MEDC (Australia) and a LEDC. Furthermore, data from a rural and an urban state in Mexico were selected to investigate the impact varying of degrees of development within the same country.

The rural and urban Mexican states were selected on the basis of population density and incidence of ascariasis. Distrito Federal (DF) is the most densely populated state with 5,896 inhabitants km^{-2}, nearly 9 times more densely occupied than the next. It presented one of the lowest incidences of *Ascaris* infection in Mexico. Of the 13 least densely populated states, those with less than 40 inhabitants km^{-2}, Chiapas was selected as the rural representative as it has one of the highest reported incidence of ascariasis. A summary of these results is shown in Table 5.4. A complete listing is in Appendix A.

Table 5.4. Population density and incidence of ascariasis in two states in Mexico (compiled from INEGI, 2005 and Secretaría de Salud, 2008)

State	Population density [a] (inhabitants km^{-2})	Incidence of ascariasis [b] (per 10^5 inhabitants)
Distrito Federal	5896.5	30.4
Chiapas	28.6	223.9

[a] 2005 [b] 2008

5.4. Treatment methods and harvest period considered

A conventional wastewater and sludge treatment sludge train was the basis for this analysis and included the following steps:

- Primary sedimentation with a 2-hour detention time (Metcalf and Eddy Inc., 2004)
- Activated sludge process (ASP), followed by secondary clarification

- Mesophilic anaerobic digestion (MAD) with a mean retention period of 12 days at 35°C ±3°C followed by storage for 14 days.

- Mechanical dewatering

- 10 month harvest period: the minimum waiting period after application of enhanced-treated biosolids to grow vegetable and salad crops in the UK (ADAS, 2001). Within the context of this investigation, this provided a conservative waiting period estimate in view that if conventionally-treated biosolids are applied, these periods extend to 12 and 30 months for vegetable and salad crops respectively.

It was assumed that pathogen inactivation only occurred during ASP, MAD and the harvest period.

5.5. Population and wastewater flow data

The population and wastewater flow data used to calculate pathogen concentrations in raw wastewater for each location in the investigation are shown in Table 5.5.

Table 5.5. Population and wastewater flow data for Victoria, Australia; Distrito Federal, Mexico and Chiapas, Mexico.

Location	Population	Wastewater flow rate (ms^{-1})
Victoria, Australia	5,204,826[a]	18.38[c]
Distrito Federal, Mexico	8,836,045[b]	3.12[d]
Chiapas, Mexico	4,460,013[b]	1.36[d]

[a] ABS (2008)
[b] INEGI (2005); Secretaría de Salud (2008)
[c] WSAA *et al.* (2009); Madden, C. (2010)
[d] CONAGUA (2010)

5.6. Calculation of pathogen concentration in raw wastewater from disease incidence data

The effect of disease frequency on the risk to human health was examined using disease incidence data published by relevant health authorities. The source for Australian infection rates was OzFoodNet, (OzFoodNet, 2009) the foodborne illness surveillance system instituted by the Australian Government Department of Health and Ageing in 2000. In Mexico, infection rates are compiled by the Ministry of Health (Secretaría de

Salud, 2008) and published by the Single Information System for Epidemiological Surveillance (known as SUIVE, Sistema Único de Información para la Vigilancia Epidemiológica).

Disease incidence and population data were used to calculate the number of infected individuals. This, along with excretion rates given in Table 5.6, was used to estimate the excretion rate of the infected population. It was subsequently converted into pathogen input to wastewater using the pathogen excretion rates detailed in Table 4.1. Finally, the pathogen concentration in raw wastewater was obtained from wastewater flow rates and pathogen inputs. This procedure is summarized graphically in Figure 5.1.

Table 5.6. Daily excretion rates in LEDCs and MEDCs (adapted from Feachem, 1983)

Age group	Daily excretion rate (g capita $^{-1}$d^{-1})		
	MEDC	LEDC urban	LEDC rural
Under 15 years old	75	125	175
Over 15 years old	150	250	350

Assumptions relevant to this part of the investigation include:

- **Source of pathogens**. As the man–sludge–soil–crop–man infection pathway was being studied, only reported infections were considered as source of pathogens. Therefore, other pathogen sources, such as animal abattoirs and excretion by asymptomatic carriers in the human population, were not considered.
- **Notification of infection**. Enteric disease symptoms range from asymptomatic to acute so it is likely some of the asymptomatic and milder infections will go unreported. However, asymptomatic individuals are likely to excrete lower numbers of pathogens than symptomatic individuals

- **Disease seasonality.** Disease incidence data was reported on an annual basis, which does not show seasonal variation of diseases.

- **All faeces from infected population enter wastewater**. Faeces from infected infants are more likely to be disposed of as solid waste rather than enter wastewater.

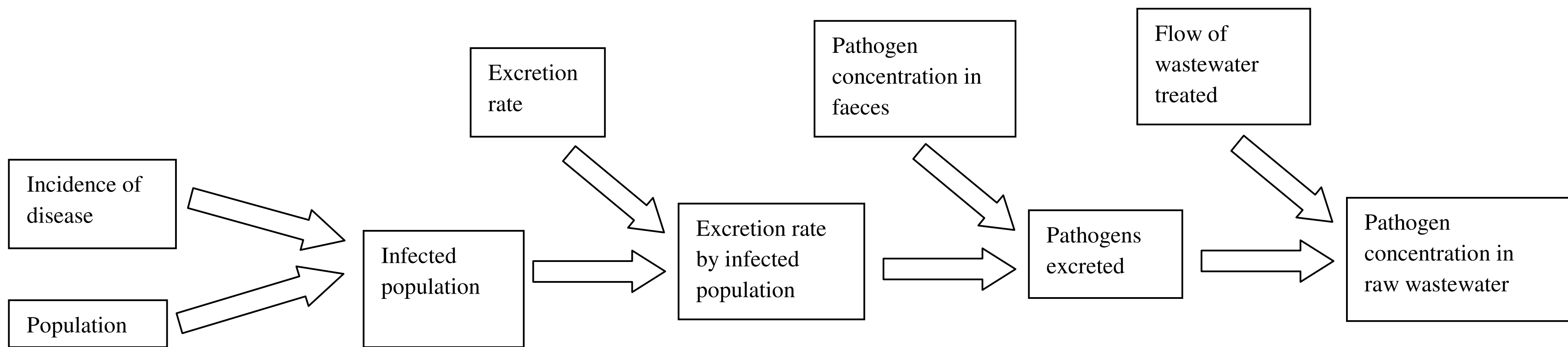


Figure 5.1. Flow chart showing the approach used to estimate the pathogen concentration in raw wastewater from information about the incidence of disease and other data.

5.7. Estimation of pathogen concentration in raw sludge

The pathogen loading in raw sludge is estimated using the first type of event tree developed by Gale (2002; 2003), an example of which is depicted in Fig. 5.2. Reported partitions of the pathogen (in this case, *Salmonella* spp.) throughout the wastewater treatment train were used to estimate the pathogen concentration in raw sludge.

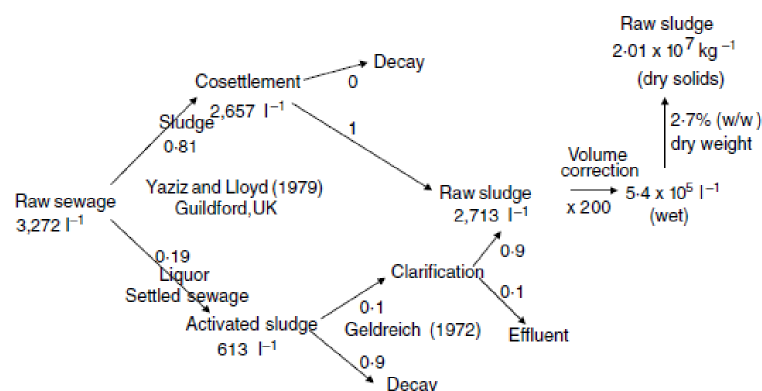


Figure 5.2. Event tree for partitioning of *Salmonella*s into raw sewage sludge at WWTP (Gale, 2003; p. 37)

Pathogens in raw wastewater are partitioned into primary sludge and settled sewage. The latter portion proceeds to the activated sludge step, and subsequently, secondary sedimentation (clarification). Pathogen concentrations in primary and secondary sludges are compiled as raw sludge. It is estimated that 200L of wastewater generate 1L of wet sludge, so a volume correction factor of 200 is applied (Gale, 2002). The resulting wet sludge concentration is converted to dry weight using the assumed dry solids content of wet sludge. This procedure is summarised graphically in Figure 5.3. Assumptions applied at this stage include:

- **Pathogen decay.** Pathogen decay only occurs during the activated sludge process (ASP); none occurs in primary or secondary sludge.
- **Volume correction.** Wastewater treatment separates the bulk of the water and sludge fractions, resulting in a significant difference in volume. Consequently, the pathogen concentration increases and therefore a volume correction factor is required. Gale estimated 1L raw sludge results from 100L to 200L of raw sewage. Thus, volume correction factors of 100 and 200 were used in 2002 and 2003, respectively. A volume factor of 200 was used for the purposes of this

investigation as it is both the more recent and more conservative of the two reported values.

- **Sludge density.** 1L of sludge has a mass of 1kg.

- **Dry solids content.** Gale (2002. 2003) and Horan (2004) reported the dry solids content of raw sludge as: 2.7% w/w and 3.3%w/w respectively. The average value of 3.0%w/w dry solids was used in this investigation.

Partitions in primary and secondary sedimentation, as well as activated sludge process (ASP) removal factors were obtained from the reported values listed in Tables 4.3 to 4.5. The risk assessment was performed under "typical" and "worst-case" conditions. Mean removals and partitions were used as typical values. Where more than one removal was reported, the poorest (that is, the one resulting in the greatest concentration of pathogens in sludge) was taken to represent the conservative worst-case. Typical and worst-case removals are summarised in Table 5.7

Table 5.7. Typical and worst-case removals in wastewater treatment processes reported previously in Chapter 4.2

Pathogen	Primary sedimentation (% organisms transferred into sludge)		Activated sludge process (% organisms inactivated)		Secondary sedimentation (% organisms transferred into sludge)	
	Typical	Worst-case	Typical	Worst-case	Typical	Worst-case
Viruses						
HAV	34.6	70	90	90	90	90
Rotavirus	34.6	70	90	90	90	90
Bacteria						
Campylobacter	70.9	78	90	90	90	90
Salmonella	65.5	81	90	90	90	90
Shigella	65.5	65.5	90	90	92.6	83.8
Protozoan (oo)cysts						
***Cryptosporidium* oocysts**	55.8	99	90	90	90	90
***Giardia* cysts**	36.7	50	90	90	86.5	77.6
Helminth ova						
Ascaris	90	90	99.7	99.7	99.74	99.74
Taenia	94	98	99	99	99	99

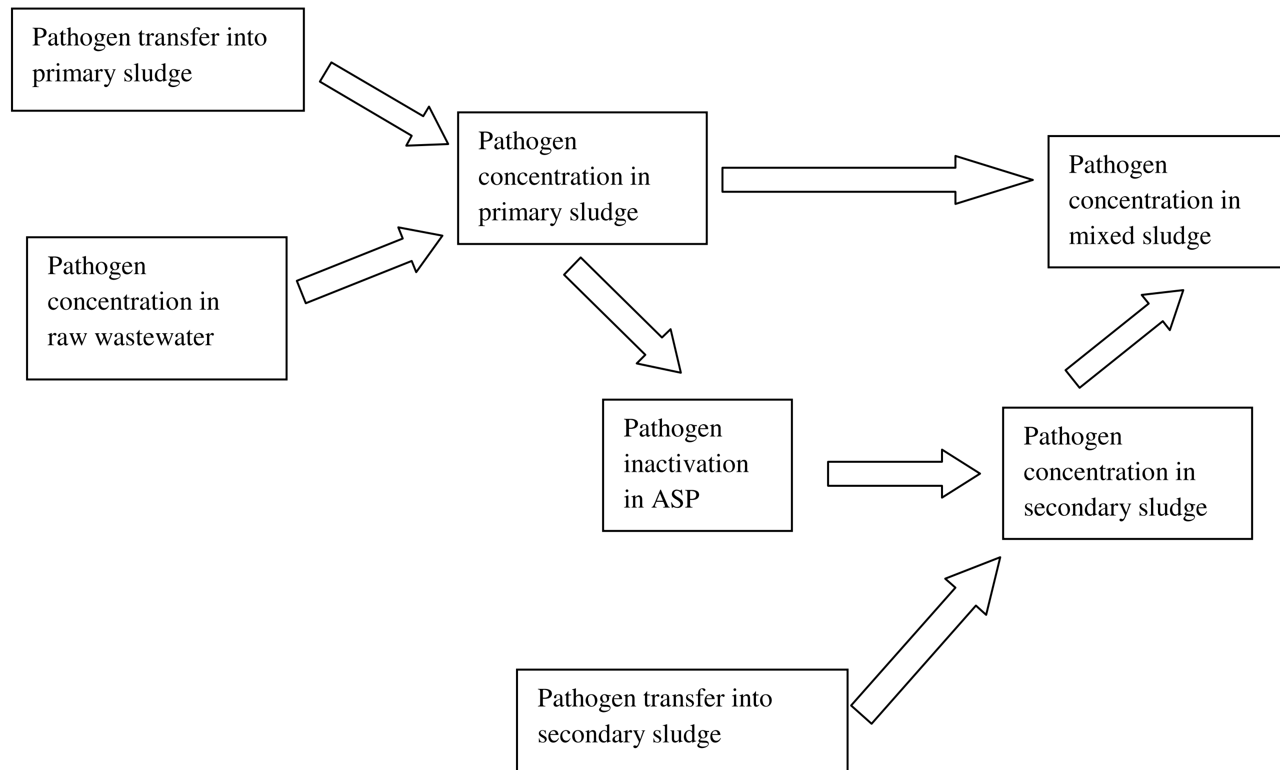


Figure 5.3. Flow chart showing the approach used to estimate the number of pathogens in sewage sludge from their concentration in raw wastewater.

5.8. Calculation of pathogen crop loading

A second event tree was developed by Gale (2002; 2003) to show pathogen partitioning in sludge treatment, storage and recycling onto land (Figure 5.4) to estimate the pathogen crop loading at point of harvest. Pathogen decay takes place during mesophilic anaerobic digestion (MAD) and the waiting period between land application and harvest. Pathogen concentrations are partitioned due to dilution in soil and probability of contact with the crop's surface.

In the present investigation the effects of conventional treatment are being studied, therefore, the enhanced treatment step shown in Figure 5.4 was omitted from the procedure. A summary of the procedure followed in the present investigation is presented in Figure 5.5.

Typical and worst-case pathogen decay values for mesophilic anaerobic digestion (MAD) and in soil from Table 4.8 are summarized in Table 5.8.

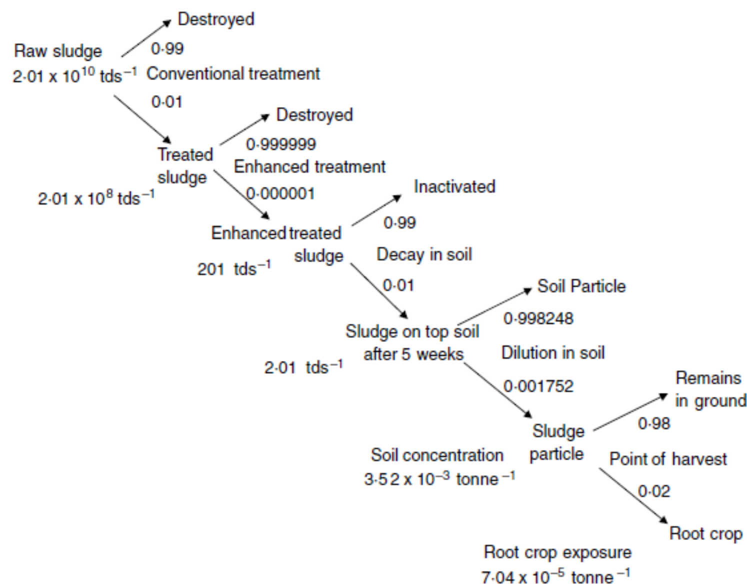


Figure 5.4. Event tree for transmission of *Salmonella*s in sewage sludge to root crops (Gale, 2003; p. 39)

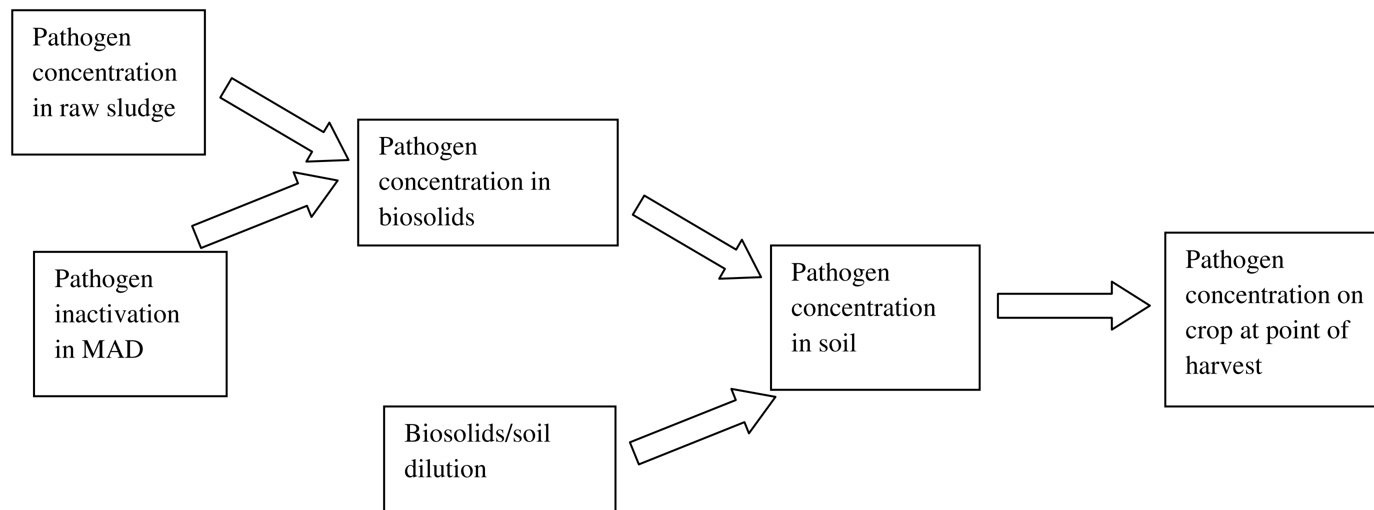


Figure 5.5. Flow chart showing the approach used to estimate the number of pathogens in crops from their concentration in raw sludge.

Table 5.8. Mean and worst-case removals in sewage sludge treatment and decay in soil

Pathogen	Mesophilic anaerobic digestion (% organisms inactivated)		10-month decay in soil (log inactivation)	
	Typical	Worst-case	Typical	Worst-case
Viruses				
HAV	71.6	8.8	31.2	1.7
Rotavirus	82.5	51	25	13.5
Bacteria				
***Campylobacter* spp.**	84.6	56.3	45.5	45.5
***Salmonella* spp.**	98.7	97.2	18.3	18.3
***Shigella* spp.**	98	96.1	6.5	6.5
Protozoan (oo)cysts				
***Cryptosporidium* spp.**	83.8	49.9	3.4	3
Giardia lamblia	99.8	0	10	10
Helminth ova				
Ascaris	13.3	0	0.33	0.33
Taenia	99	99	6.7	4

Simplifying assumptions reported by Gale (2002) also apply to this investigation:

- **Pathogen leaching.** It is assumed no pathogens are leached after MAD.
- **Other pathogen inputs to land.** The only pathway considered is sewage to land; pathogen input due to septic tank overflow, or animal and human defecation are not considered. Only soil adhered to crops is the source of pathogens arising from biosolids.
- **Partitioning of pathogens from soil to crop.** 2% of the harvested crop's mass is soil.
- **Biosolids application onto land.** Sludge is homogenously ploughed or injected into soil to a 0.25m depth.
- **Influence of climate on pathogen decay in soil.** Seasonal variations in pathogen decay in soil over the harvest period were not taken into account.

5.9. Calculation of individual exposure

Annual exposure to pathogens adhered to crops was calculated using the simplifying assumptions proposed by Gale (2002) concerning handling and consumption of vegetable crops:

- **Washing.** 90% of any pathogens adhered onto the crop are washed away.
- **Consumption of raw vegetable crops.** A person consumes 70g of vegetable crops daily. 50% of the crop is consumed raw or unpeeled; therefore 35g d^{-1} of uncooked vegetable crop is consumed per capita.

These assumptions equate vegetable handling and consumption practices in Mexico and Australia to allow a comparison of risk of infection to be made.

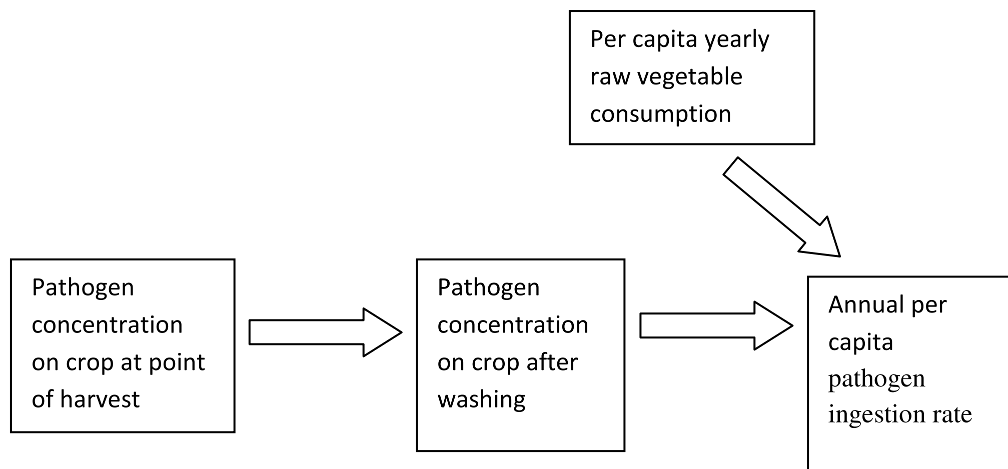


Figure 5.6. Flow chart showing the approach used to calculate annual exposure to pathogens per capita.

5.10. Calculation of annual risk of infection *per capita*

The final RA stage is shown in Figure 5.7. Annual per capita pathogen ingestion (the dose) and published dose-response data (Chapter 3.5) were used to estimate the risk of infection posed by that particular exposure.

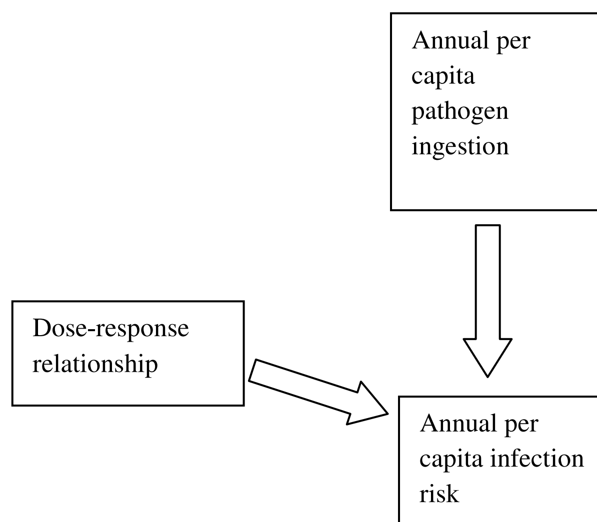


Figure 5.7. Flow chart showing the approach used to calculate annual risk of infection per capita.

The following assumptions were made at this stage:

- **Pathogen infectivity.** It was assumed that pathogens are viable and equally infectious (Haas, 1996)
- **Previous exposure.** Pathogens act independently so that past exposure to pathogens does not affect a host's risk of infection (Haas *et al.* 1993), therefore acquired immunity is not accounted for.
- **Arithmetic means adequately summarize pathogen exposure data.** Haas (1996) concluded that, for risk assessment purposes, pathogen exposures were adequately condensed by arithmetic means.

Due to lack of data for the dose-response relationships of Hepatitis A virus (HAV), *Shigella* and *Taenia*, known models were used. HAV and *Shigella* were modelled using the Rotavirus and *E. coli* relationships, respectively. In the case of *Taenia*, it was assumed that one ovum or cysticercus caused one infection, which is conservative.

5.11. Worked example for *Giardia lamblia* in Victoria

a) Daily excretion

Age group	Population	No. infections	Excretion rate (g $p^{-1}d^{-1}$)	Daily excretion (g d^{-1})
Under 15	281,287	430	75	32,250
Over 15	4,223,539	762	150	114,300
			Total	146,550

b) Pathogen excretion rate:

Multiply by pathogen density in faeces ($3.2x10^6$ /g^{-1}faeces)

(146,550 g d^{-1})($3.2x10^6$ /g^{-1}faeces)

= $4.73x10^{11}$ d^{-1}

c) Concentration of pathogens in raw wastewater:

Use flow of wastewater treated in Victoria = 18.38 m^3s^{-1}

$$\frac{4.73x10^{11} cysts d^{-1}}{(1000 L m^{-3})(3600 s h^{-1})(24 h d^{-1})(18.38 m^3 s^{-1})}$$

= $2.98x10^2$ L^{-1}

d) Concentration of pathogens in raw sludge

Partitions and decay during wastewater treatment are shown in Figure 5.8.

e) Pathogen crop loading at point of harvest

Partitions and decay during sludge treatment are shown in Figure 5.9.

f) Pathogen concentration on washed vegetables grown in biosolids-amended soil:

Washing removes 1 log of soil:

= ($6.34x10^{-15}$ cysts g^{-1} crop) (0.1)

= $6.34x10^{-16}$ cysts g^{-1} crop

g) Pathogen ingestion:

($6.34x10^{-16}$ cysts g^{-1} crop)(35g crop p^{-1} y^{-1})(365 d y^{-1})

$= 8.10x10^{-12}$ cysts p^{-1} y^{-1}

h) Annual individual infection risk

As shown in Table 3.5, the dose-response relationship for giardiasis infection is exponential (3. 1). The parameter *r* is 0.0189 for *Giardia.*

$$p = 1 - e^{-rN}$$

$p_{Giardia} = 1 - e^{-0.0198(8.10x10\text{^}-12)}$

$= 1.60x10^{-13}$ pppy

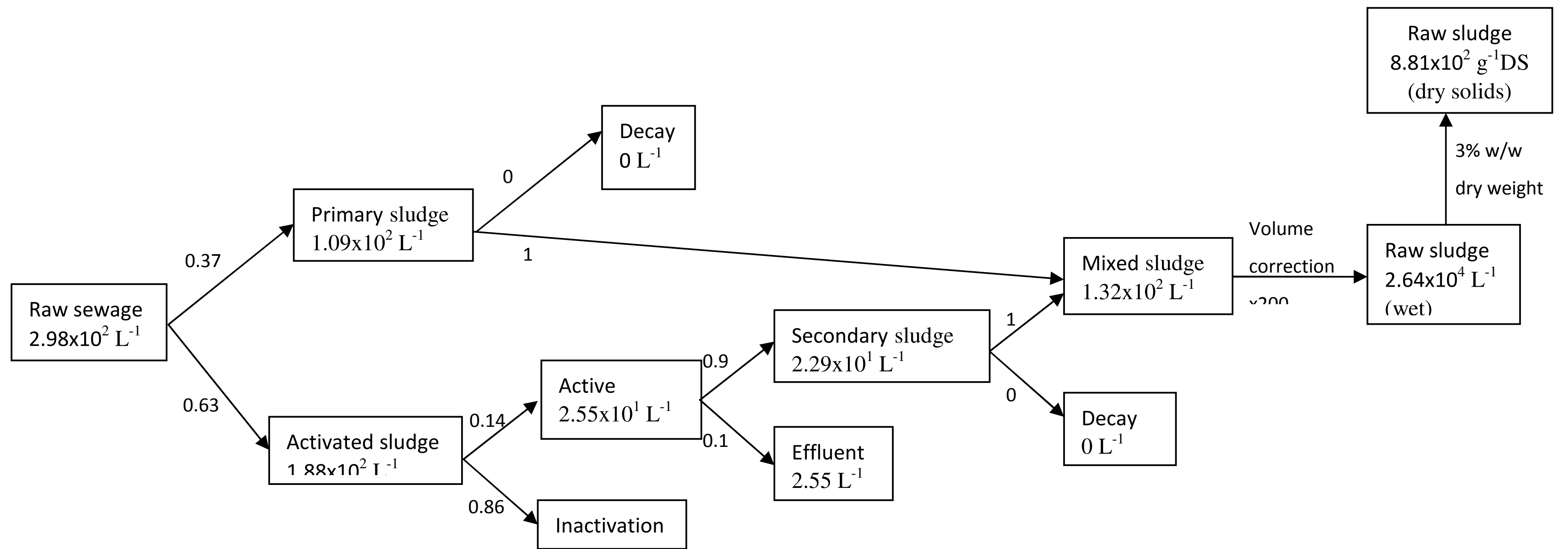


Figure 5.8. Event tree for occurrence of *Giardia lamblia* cysts in wastewater and sludge

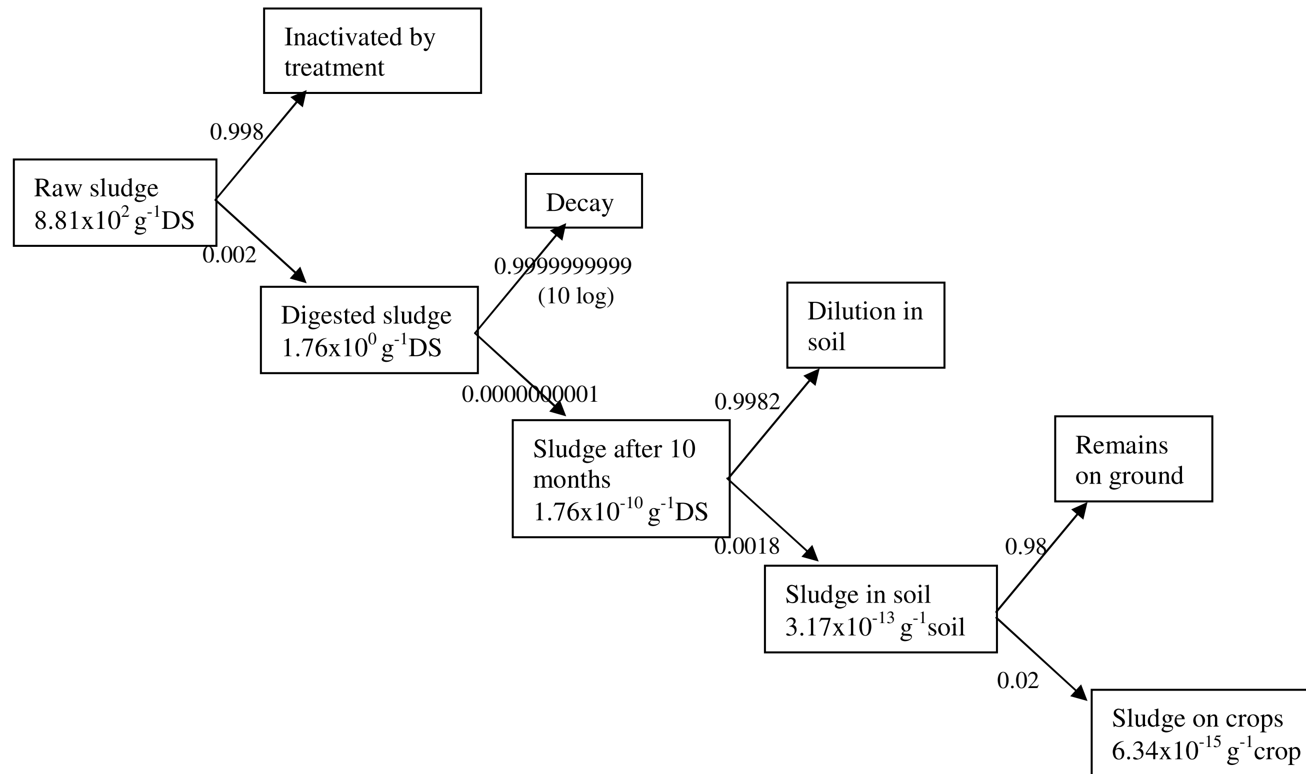


Figure 5.9. Event tree for occurrence of *Giardia lamblia* cysts in sludge, biosolids and biosolids-amended soil

6. Results

To assess the validity of the model at estimating pathogen concentrations, the model output was compared with literature values (Chapter 6.1). Resulting annual risks of infection were ranked and discussed (Chapter 6.2). Differences in model outputs for crop loadings and annual risk of infection under typical and worst-case removals were compared in Chapter 6.3. Finally, as ascariasis and taeniasis are not notifiable diseases in Victoria, sensitivity analysis was conducted to assess the risk of infection by *Ascaris* and *Taenia* ova in Victoria and presented in Chapter 6.4.

6.1. Model validity based on literature values

The ranges of reported pathogen concentrations presented in Tables 4.2, 4.6, 4.7 and 4.9 are summarised in Table 6.1. To assess the validity of the model, these data were compared with the model output. A model output value was considered to be consistent with published data if it lay within one order of magnitude of the reported value. The outputs of the model are shown in Table 6.2.

6.1.1. General

Overall, the modelled concentrations of pathogens in treated biosolids (Table 6.2) were consistent with available published data in Table 6.1 including the distinction between these values in LEDCs and MEDCs, where possible. Overestimations for some pathogen densities in treated biosolids including *Campylobacter* and rotavirus, suggest the modelled inactivation of these organisms is conservative. The model underestimated *Shigella* concentrations in raw sludge, but was consistent with the values reported for treated sludge.

Lack of data prevented verification of HAV and *Taenia* in sludge.

Table 6.1. Ranges of pathogen concentrations reported in the literature cited in chapter 4. Where possible, a distinction between MEDCs and LEDCs has been made.

Pathogen	Raw wastewater (L^{-1})	Treated wastewater (L^{-1})	Raw sludge (g^{-1}DS)	Treated sludge (g^{-1}DS)
Viruses				
HAV	10^7	--	--	--
Rotavirus	$10^1 – 10^4$	$<10^0 – 10^3$ (MEDC)	--	$10^1 – 10^2$
Bacteria				
Campylobacter	10^4 (MEDC)	10^1 (MEDC)	$10^5 – 10^{11b}$	10^2
Salmonella	10^3 (MEDC) $10^6 – 10^9$ (LEDC)	--	$10^2 – 10^{7b}$ (MEDC) $10^6 – 10^8$ (LEDC)	$10^{-1} – 10^3$
***Shigella* sp.**	$10^1 – 10^4$	--	10^7	10^1
Protozoan (oo)cysts				
***Cryptosporidium* oocysts**	$10^{-1} – 10^4$	$<10^0 – 10^2$ (MEDC)	$10^{-1} – 10^{2b}$	$10^0 – 10^2$
***Giardia* cysts**	$10^1 – 10^5$	$10^{-1} – 10^0$ (MEDC) 10^2 (LEDC)	$10^{-1} – 10^3$ (MEDC) $10^2 – 10^4$ (LEDC)	$10^{-2} – 10^3$
Helminth ova				
***Ascaris* ova**	$0 – 10^3$ (LEDC)	10^1 (LEDC)	$10^{-1} – 10^{1a}$ (MEDC) $10^0 – 10^{2a}$ (LEDC)	$10^{-1} – 10^{1a}$ (MEDC) $10^0 – 10^{2a}$ (LEDC)
***Taenia* ova**	10^1 (LEDC)	10^0 (LEDC)		

[a] Reported as helminth ova

[b] Values reported in a model (Gale, 2002) are upper limit

-- No data

Table 6.2.Model output for pathogen concentrations in raw and treated wastewater, raw and treated sludge, crop loading and estimated annual risk of infection from ingestion of crops grown in biosolids-amended soil.

Pathogen	Location	Incidence (per 10^5 inhabitants)	Pathogen concentration					
			Raw wastewater (L^{-1})	Treated wastewater (L^{-1})	Raw sludge (g^{-1}DS)	Treated sludge (g^{-1}DS)	Crop loading (g^{-1}crop)	Annual risk of infection (pppy)
Viruses								
HAV	Victoria	0.88	$1.4x10^6$	$8.0x10^3$	$3.4x10^6$	$2.4x10^5$	$5.5x10^{-31}$	$<10^{-27}$
	DF, Mexico	16.87	$3.3\ x10^8$	$1.9x10^6$	$7.9x10^8$	$5.7x10^7$	$1.3x10^{-28}$	$<10^{-25}$
	Chiapas, Mexico	10.70	$3.1x10^8$	$1.8x10^6$	$7.5x10^8$	$5.4x10^7$	$1.2x10^{-28}$	$<10^{-25}$
Rotavirus	Victoria	0.5	$5.4x10^5$	$3.1x10^3$	$1.3x10^6$	$3.2x10^5$	$1.2x10^{-24}$	$<10^{-21}$
	DF, Mexico	0.03	$3.4x10^5$	$1.9x10^3$	$8.2x10^5$	$2.0x10^5$	$7.2x10^{-25}$	$<10^{-21}$
	Chiapas, Mexico	2.58	$4.5x10^7$	$2.6x10^5$	$1.1x10^8$	$2.7x10^7$	$9.5x10^{-23}$	$<10^{-20}$
Bacteria								
Campylobacter [a]	Victoria	110.17	$1.1x10^5$	$1.2x10^1$	$5.0x10^5$	$7.7x10^4$	$2.8x10^{-45}$	$<10^{-41}$
***Salmonella*, not including typhoid fever**	Victoria	28.30	$5.7x10^3$	$2.0x10^1$	$2.6x10^4$	$3.5x10^2$	$5.8x10^{-21}$	$<10^{-21}$
	DF, Mexico	19.39	$7.1x10^4$	$2.5x10^2$	$3.3x10^5$	$4.4x10^3$	$7.9x10^{-20}$	$<10^{-19}$
	Chiapas, Mexico	421.57	$2.5x10^6$	$8.7x10^3$	$1.2x10^7$	$1.6x10^5$	$2.8x10^{-18}$	$<10^{-18}$

[a] Incidence data unavailable for Mexico

Table 6.2 *continued*. Individual annual risk of enteric disease infection from ingestion of crops grown in biosolids-amended soil.

Pathogen	Location	Incidence (per 10^5 inhabitants)	Pathogen concentration: Raw wastewater (L^{-1})	Treated wastewater (L^{-1})	Raw sludge (g^{-1}DS)	Treated sludge (g^{-1}DS)	Crop loading (g^{-1}crop)	Annual risk of infection (pppy)
***Salmonella*, including typhoid fever**	Victoria	28.70	$6.5x10^{3}$	$2.2x10^{1}$	$3.0x10^{4}$	$4.0x10^{2}$	$7.2x10^{-21}$	$<10^{-18}$
	DF, Mexico	21.14	$7.8x10^{4}$	$2.7x10^{2}$	$3.6x10^{5}$	$4.8x10^{3}$	$8.7x10^{-20}$	$<10^{-16}$
	Chiapas, Mexico	493.13	$3.0x10^{6}$	$1.0x10^{4}$	$1.4x10^{7}$	$1.8x10^{5}$	$3.3x10^{-18}$	$<10^{-15}$
Shigella	Victoria	1.60	$2.0x10^{2}$	$3.7x10^{-1}$	$8.9x10^{2}$	$1.8x10^{1}$	$2.0x10^{-10}$	$1.3x10^{-9}$
	DF, Mexico	1.47	$2.8x10^{3}$	$5.2x10^{0}$	$1.2x10^{4}$	$2.5x10^{2}$	$2.7x10^{-9}$	$1.9x10^{-8}$
	Chiapas, Mexico	30.38	$7.7x10^{4}$	$1.5x10^{2}$	$3.5x10^{5}$	$6.9x10^{3}$	$7.7x10^{-8}$	$5.2x10^{-7}$
Protozoan (oo)cysts								
Cryptosporidium [a]	Victoria	21.35	$2.0x10^{3}$	$9.6x10^{0}$	$7.9x10^{3}$	$1.4x10^{3}$	$3.0x10^{-5}$	$6.0x10^{-5}$
Giardia	Victoria	22.90	$3.0x10^{2}$	$3.4x10^{0}$	$8.8x10^{2}$	$1.8x10^{0}$	$6.3x10^{-15}$	$1.6x10^{-13}$
	DF, Mexico	25.72	$5.3x10^{3}$	$6.1x10^{1}$	$1.6x10^{4}$	$3.1x10^{1}$	$1.1x10^{-13}$	$2.8x10^{-12}$
	Chiapas, Mexico	38.32	$1.2x10^{4}$	$1.4x10^{2}$	$3.5x10^{4}$	$6.9x10^{1}$	$2.5x10^{-13}$	$6.3x10^{-12}$

[a] Incidence data unavailable for Mexico

Table 6.2 *continued*. Individual annual risk of enteric disease infection from ingestion of crops grown in biosolids-amended soil.

Pathogen	Location	Incidence (per 10^5 inhabitants)	Pathogen concentration					Annual risk of infection (pppy)
			Raw wastewater (L^{-1})	Treated wastewater (L^{-1})	Raw sludge (g^{-1}DS)	Treated sludge (g^{-1}DS)	Crop loading (g^{-1}crop)	
<u>Helminth ova</u>								
Ascaris [b]	DF, Mexico	30.42	$1.7x10^{1}$	$1.7x10^{0}$	$1.1x10^{2}$	$9.9x10^{1}$	$1.7x10^{-3}$	$1.1x10^{-1}$
	Chiapas, Mexico	223.85	$1.9x10^{2}$	$1.9x10^{1}$	$1.3x10^{3}$	$1.1x10^{3}$	$1.9x10^{-2}$	$2.8x10^{-1}$
***Taenia* ova** [b]	DF, Mexico	0.00	n/a	n/a	n/a	n/a	n/a	n/a
	Chiapas, Mexico	1.50	$1.3x10^{0}$	$8.1x10^{-2}$	$8.9x10^{0}$	$8.9x10^{-2}$	$1.6x10^{-11}$	$2.1x10^{-8}$
***Taenia* ova and cysticerci** [b]	DF, Mexico	0.24	$2.0x10^{-1}$	$1.2x10^{-2}$	$1.3x10^{0}$	$1.3x10^{-2}$	$2.4x10^{-12}$	$3.0x10^{-9}$
	Chiapas, Mexico	1.55	$1.4x10^{0}$	$8.4x10^{-2}$	$9.3x10^{0}$	$9.3x10^{-2}$	$1.7x10^{-11}$	$2.2x10^{-8}$

[b] Incidence data unavailable for Victoria

n/a not applicable due to disease incidence being zero in Distrito Federal

6.1.2. Comparison of reported values to model output for individual pathogens

Viruses

HAV: The model output for HAV concentration in wastewater (10^6 – 10^8 PFU L^{-1}; Table 6.2) agreed with the reported value of 10^7 PFU L^{-1} (Table 6.1). Data for HAV concentrations in biosolids are scarce.

Rotavirus: Victoria and Distrito Federal (DF) rotavirus wastewater and effluent concentrations were 10^5 and 10^3 PFU L^{-1} respectively (Table 6.2), which were consistent with the upper end of the range of reported values (Table 6.1). Rotavirus concentrations in biosolids were overestimated by 3 to 6 orders of magnitude, suggesting the model was conservative with respect to rotavirus removal in MAD. The lower end of the range of reported rotavirus removals by MAD (Table 4.8) may be lower than in practice, and therefore, the use of these values lead to overestimated pathogen numbers.

Bacteria

Campylobacter**:** The model gave raw wastewater and effluent *Campylobacter* concentrations that agreed with the reported concentrations of 10^4 and 10^1 PFU L^{-1} respectively (Tables 6.1 and 6.2). The modelled *Campylobacter* concentration in raw sludge is consistent with the lower end of the reported range of 10^5 – 10^{11} g^{-1}DS (the latter value is itself a modelled result and may be conservative, explaining why it is considerably higher). *Campylobacter* densities in treated biosolids were overestimated by two orders of magnitude, suggesting the model was overly conservative with regards to *Campylobacter* inactivation by MAD.

Salmonella**:** Reported *Salmonella* densities (Table 6.1) span a variety of countries, allowing for verification of the modelled output under different socioeconomic contexts. Victorian *Salmonella* concentrations agreed with the published values for MEDCs. The output for Chiapas also agreed with those values reported for LEDCs, which are 2-3 orders of magnitude greater than MEDC values. Results for Distrito Federal fell between MEDC and LEDC densities. The incorporation of typhoid incidence data into the model did not significantly affect the model – results for *Salmonella* are similar regardless of whether typhoid fever incidence data was included or not.

***Shigella*:** Model output of raw wastewater and treated biosolids concentrations of *Shigella* (Table. 6.2) agreed with the range of data reported (Table 6.1). Chiapas gave a result higher than reported concentrations but presumably this was due to the significantly greater incidence of shigellosis (30 infections per 10^5 inhabitants in Chiapas, compared to <2 in DF and Victoria; see Table 6.2). Due to the lack of data on *Shigella* inactivation during the activated sludge process, values reported for *E. coli* and faecal coliforms were employed instead. However, the model underestimated the reported value for *Shigella* concentration in raw sludge by at least three orders of magnitude, indicating this data replacement may not be appropriate. A reason for this might be the fact that *Shigella* infections may not always be diagnosed as they can resemble symptoms due to other pathogens.

Protozoan cysts and oocysts

***Cryptosporidium* oocysts:** The model output for *Cryptosporidium* oocyst concentrations in Victorian wastewater and sludge (Table 6.2) were consistent with published values (Table 6.1).

***Giardia* cysts:** Predicted *Giardia* densities (Table 6.2) fall within reported ranges for wastewater, raw sludge and treated biosolids, and were consistent with differences between MEDCs and LEDCs. Modelled *Giardia* density in Victorian effluent (10^2 cysts L^{-1}; Table 6.2) was higher than published MEDC values (10^{-1} – 10^0 cysts L^{-1}; Table 6.1). Additionally, modelled Mexican values were on the higher end of the published ranges, suggesting *Giardia* cyst inactivation during the activated sludge process (ASP) was overly conservative in the model.

Helminth ova

***Ascaris* ova:** The model output for *Ascaris* ova concentrations in wastewater and sludge under Mexican conditions (Table 6.2) agreed with the values reported in the literature (Table 6.1). Values for Chiapas (rural Mexico) were consistently higher than those for DF (urban Mexico).

***Taenia* ova:** Modelled Mexican *Taenia* ova densities in raw wastewater and effluent (Table 6.2) underestimated published data values (Table 6.1) by one and two orders of magnitude, suggesting a considerable contribution of ova from sources other than the infected population, including asymptomatic carriers. As *Taenia*sis produces only mild

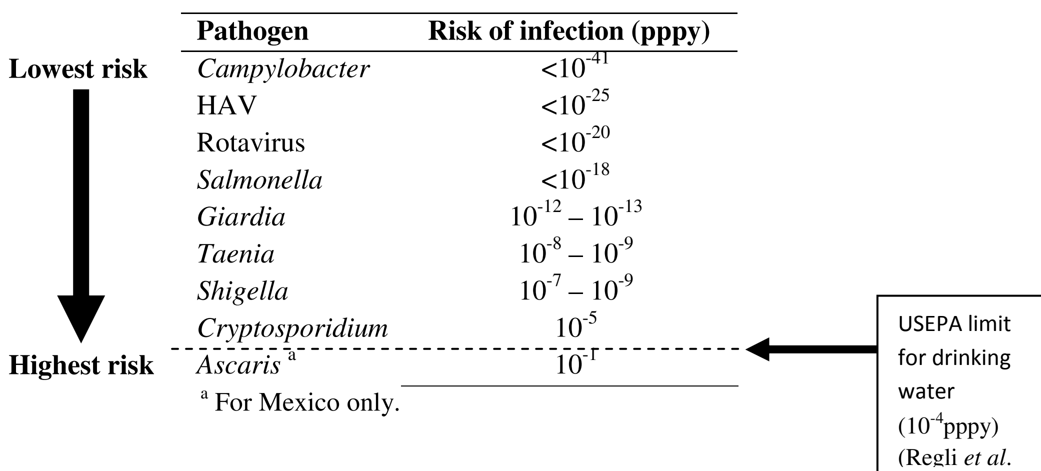
abdominal symptoms (CDC, 2009), this disease is likely to go unreported unless severe cysticercosis is experienced. Data on *Taenia* concentrations in wastewater and sludge are scarce.

6.2. Risk of infection

This QMRA model predicted very low annual risks of infection by ingestion of vegetable crops grown in conventionally-treated biosolids-amended soil for the majority of the pathogens included in the study. The ranking in Table 6.3 shows risks modelled for *Campylobacter*, HAV, rotavirus, *Salmonella*, *Giardia, Taenia* and *Shigella* were below the 10^{-4}pppy limit for drinking water proposed by USEPA (Regli *et al.,* 1991) by at least three orders of magnitude.

Risk of infection from *Cryptosporidium* oocysts was only one order of magnitude below the limit, highlighting it for further study into its decay in soil, which is likely to be affected by climate; faster decay rates have been reported at warmer temperatures (Olson *et al.,* 1999).

Table 6.3. Risk of infection of enteric pathogens from the agricultural reuse of biosolids.



Pathogen	Risk of infection (pppy)
Campylobacter	$<10^{-41}$
HAV	$<10^{-25}$
Rotavirus	$<10^{-20}$
Salmonella	$<10^{-18}$
Giardia	$10^{-12} – 10^{-13}$
Taenia	$10^{-8} – 10^{-9}$
Shigella	$10^{-7} – 10^{-9}$
Cryptosporidium	10^{-5}
Ascaris [a]	10^{-1}

[a] For Mexico only.

The physical resilience of *Ascaris* ova (Strauch *et al.,* 1991; Jimenez, 2007), and the resulting low inactivation throughout the conventional treatment train, led to a high risk of infection. However, this pathogen is not prevalent in MEDC regions such as Australia (EPAV, 2004). Sensitivity analysis in Chapter 6.4 explored the impact of

ascariasis prevalence on risk of infection to assess the relevance of this problem in Australia.

Interestingly, risk of ascariasis infection is similar for both DF (1.1×10^{-1} pppy) and Chiapas (2.8×10^{-1}pppy): in both cases on the order of 10^{-1}pppy in spite of the tenfold difference in crop loading (10^{-3} and $10^{-2}g^{-1}$crop in DF and Chiapas, respectively). These results correspond to 10^{0} and 10^{1} ova per person per year and suggest that, **at these doses**, risk of infection is not significantly affected by a tenfold difference in exposure.

6.3. Worst-case results

6.3.1. General overview of impact of worst-case situation

As explained in sections 5.1, 5.7 and 5.8, this QMRA was performed using typical and worst case values for pathogen partitions and inactivation (Table 6.4). Risks of infection from bacteria under worst-case conditions were 0 to 1 orders of magnitude greater than under typical conditions, but remained well under the USEPA drinking water limit of 10^{-4}pppy. Worst-case conditions had a slightly greater impact on the risk from *Giardia* cysts, but still remained below 10^{-4}pppy. The order of magnitude of the risk posed by helminth ova remained the same than under typical removals.

Worst-case removals had a significant impact on risks of viral infection, presumably due to the large range of reported inactivation in soil for these microorganisms.

6.3.2. Comparison of typical and worst-case results for individual pathogens

__Viruses__

HAV: This pathogen had the greatest degree of uncertainty associated with it due to lack of data, and the greatest impact of worst-case scenario conditions. Risk of HAV infection went from being negligible ($<10^{-25}$pppy) to 10^{-1}pppy, which is well above the 10^{-4}pppy limit of risk for drinking water recommended by USEPA (Regli *et al.*, 1991). HAV decay values and dose-response behaviour was entirely modelled using data for a variety of other organisms. Furthermore, the modelled output for HAV concentrations in sludge was not verified due to lack of data. The main influence on resulting risk of

infection values appeared to be significant variation in HAV soil decay, which ranged from 1.7 log to 31.2 log under different temperature and soil type conditions (Sobsey *et al.*, 1995).

Rotavirus: Although worst-case values for risk from rotavirus infection are still under the USEPA limit for drinking water, they were significantly greater than typical values: under worst-case conditions risk of infection from rotavirus rose from $<10^{-21}$ to 10^{-9}pppy in Victoria and DF; and from $<10^{-20}$ to 10^{-7} in Chiapas.

Although risk of infection was estimated from reported rotavirus dose-response relationship, much of the removal data for rotavirus was based on published removals of viral indicators. As with, HAV, reported virus decay in soil varied over several logs: from 13.5 to 25logs.

Table 6.4. Comparison of modelled output for crop loading and annual risk of infection using mean removals and worst-case removals

Pathogen	Location	Pathogen crop loading (g^{-1}crop)		Annual risk of infection (pppy)	
		Typical values	Worst-case values	Typical values	Worst-case values
<u>Viruses</u>					
HAV	Victoria	$5.5x10^{-31}$	$1.7x10^{0}$	$<10^{-27}$	$8.9x10^{-1}$
	DF, Mexico	$1.3x10^{-28}$	$3.9x10^{2}$	$<10^{-25}$	$9.7x10^{-1}$
	Chiapas, Mexico	$1.2x10^{-28}$	$3.7x10^{2}$	$<10^{-25}$	$9.7x10^{-1}$
Rotavirus	Victoria	$1.2x10^{-24}$	$6.9x10^{-12}$	$<10^{-21}$	$5.5x10^{-9}$
	DF, Mexico	$7.2x10^{-25}$	$4.3x10^{-12}$	$<10^{-21}$	$3.4x10^{-9}$
	Chiapas, Mexico	$9.5x10^{-23}$	$5.7x10^{-10}$	$<10^{-20}$	$3.5x10^{-7}$
<u>Bacteria</u>					
Campylobacter	Victoria	$2.8x10^{-45}$	$1.4x10^{-44}$	$<10^{-41}$	$<10^{-41}$
Salmonella	Victoria	$7.2x10^{-21}$	$4.0x10^{-20}$	$<10^{-21}$	$<10^{-20}$
	DF, Mexico	$8.7x10^{-20}$	$4.8x10^{-19}$	$<10^{-19}$	$<10^{-19}$
	Chiapas, Mexico	$3.3x10^{-18}$	$1.8x10^{-17}$	$<10^{-18}$	$<10^{-17}$
Shigella	Victoria	$2.0x10^{-10}$	$1.6x10^{-9}$	$1.3x10^{-9}$	$1.1x10^{-8}$
	DF, Mexico	$2.7x10^{-9}$	$2.2x10^{-8}$	$1.9x10^{-8}$	$1.5x10^{-7}$
	Chiapas, Mexico	$7.7x10^{-8}$	$6.3x10^{-7}$	$5.2x10^{-7}$	$4.3x10^{-6}$
<u>Protozoan (oo)cysts</u>					
Cryptosporidium	Victoria	$3.0x10^{-5}$	$2.6x10^{-4}$	$6.0x10^{-5}$	$5.1x10^{-4}$
Giardia	Victoria	$6.3x10^{-15}$	$1.5x10^{-11}$	$1.6x10^{-13}$	$3.7x10^{-10}$
	DF, Mexico	$1.1x10^{-13}$	$2.6x10^{-10}$	$2.8x10^{-12}$	$6.7x10^{-9}$
	Chiapas, Mexico	$2.5x10^{-13}$	$5.8x10^{-10}$	$6.3x10^{-12}$	$1.5x10^{-8}$
<u>Helminth ova</u>					
Ascaris	DF, Mexico	$1.7x10^{-3}$	$2.1x10^{-3}$	$1.1x10^{-1}$	$1.2x10^{-1}$
	Chiapas, Mexico	$1.9x10^{-2}$	$2.4x10^{-2}$	$2.8x10^{-1}$	$3.0x10^{-1}$
Taenia	DF, Mexico	$2.4x10^{-12}$	$5.2x10^{-12}$	$3.0x10^{-9}$	$6.7x10^{-9}$

	Chiapas, Mexico	$1.7x10^{-11}$	$3.7x10^{-11}$	$2.2x10^{-8}$	$4.8x10^{-8}$

Bacteria

***Campylobacter**:* At $<10^{-41}$pppy, risk of campylobacteriosis under worst-case conditions was still the lowest out of the pathogens studied and well below the USEPA limit.

Salmonella: Under worst-case conditions, risk of salmonella infection rose by zero to one orders of magnitude. Risk from this pathogen was well under the USEPA 10^{-4}pppy limit in the three regions studied, the highest being $<10^{-17}$pppy for Chiapas under worst-case conditions and the lowest $<10^{-21}$pppy for Victoria under typical conditions.

Shigella: Risk of infection by *Shigella* rose by one order of magnitude under worst-case conditions but was still within safe limits.

Protozoan cysts and oocysts

***Cryptosporidium* oocysts:** The individual risk of infection, 10^{-5}, rose by one order of magnitude under worst-case conditions, suggesting care should be taken with *Cryptosporidium* as the limit for safe drinking water is 10^{-4}pppy (Regli *et al*., 1991).

***Giardia* cysts:** Worst-case removals caused risks of infection to rise by three orders of magnitude but they were still within safe limits as the highest risk was 10^{-8}pppy.

Helminth ova

***Ascaris* ova:** The risks of infection under typical and worst-case conditions were 10^{-1}pppy in both cases.

***Taenia* ova:** The risks of infection were largely unaffected by worst-case treatment and still several orders of magnitude below the safe limit proposed by USEPA (10^{-4}pppy).

6.4. Sensitivity analysis of infection risk of infection by *Ascaris* and *Taenia* in Victoria

Sensitivity analysis was carried out to predict the risk of infection by *Ascaris* and *Taenia* in Victoria by conducting several iterations of number of infected individuals. These are shown in Tables 6.5 and 6.6 for *Ascaris* and *Taenia* respectively.

The results of the sensitivity analysis revealed that, in order to reach the same annual risk of ascariasis infection as that in DF, 10,000 infections would need to be reported in Victoria. This corresponds to an incidence of 192 per 10^5 inhabitants in Victoria, which is far higher than the incidence of any of the notifiable foodborne diseases included in this study occurring in Victoria or DF.

Sensitivity analysis of the *Taenia* data revealed that over 100 taeniasis or cysticercosis infections would need to be reported in order for Victoria to show the same risk of these diseases as DF. These results represent 19 infections per 10^5 inhabitants, which is 80 times greater than the reported incidence of taeniasis and cysticercosis in DF.

Incidences in excess of 100 infections per 10^5 inhabitants were infrequent and only observed for four of the data in the present study: campylobacteriosis[5] in Victoria (110.17 infections per 10^5inhabitants), salmonellosis in Chiapas (421.57 and 493.13 infections per 10^5inhabitants without and with typhoid fever) and ascariasis in Chiapas (223.85 infections per 10^5inhabitants).

[5] This disease was not studied under Mexican conditions due to data scarcity.

Table 6.5. Sensitivity analysis of the impact of infections on annual risk of ascariasis infection from ingestion of crops grown in biosolids-amended soil in Victoria.

		Pathogen concentration					
Infections	**Incidence (per 10^5 inhabitants)**	**Raw wastewater (L^{-1})**	**Treated wastewater (L^{-1})**	**Raw sludge (g^{-1}DS)**	**Treated sludge (g^{-1}DS)**	**Crop loading (g^{-1}crop)**	**Annual risk of infection (pppy)**
1	0.02	8.6×10^{-4}	8.6×10^{-5}	5.7×10^{-3}	4.9×10^{-3}	8.4×10^{-8}	1.0×10^{-5}
10	0.19	8.6×10^{-3}	8.6×10^{-4}	5.7×10^{-2}	4.9×10^{-2}	8.4×10^{-7}	1.0×10^{-4}
100	1.92	8.6×10^{-2}	8.6×10^{-3}	5.7×10^{-1}	4.9×10^{-1}	8.4×10^{-6}	1.0×10^{-3}
1,000	19.21	8.6×10^{-1}	8.6×10^{-2}	5.7×10^{0}	4.9×10^{0}	8.4×10^{-5}	1.0×10^{-2}
10,000	192.13	8.6×10^{0}	8.6×10^{-1}	5.7×10^{1}	4.9×10^{1}	8.4×10^{-4}	1.0×10^{-1}

Table 6.6. Sensitivity analysis of the impact of infections on annual risk of *Taenia*sis and cysticercosis infection from ingestion of crops grown in biosolids-amended soil.

		Pathogen concentration					
Infections	**Incidence (per 10^5 inhabitants)**	**Raw wastewater (L^{-1})**	**Treated wastewater (L^{-1})**	**Raw sludge (g^{-1}DS)**	**Treated sludge (g^{-1}DS)**	**Crop loading (g^{-1}crop)**	**Annual risk of infection (pppy)**
1	0.02	8.6×10^{-4}	5.1×10^{-5}	5.7×10^{-3}	5.7×10^{-5}	1.0×10^{-14}	1.3×10^{-11}
10	0.19	8.6×10^{-3}	5.1×10^{-4}	5.7×10^{-2}	5.7×10^{-4}	1.0×10^{-13}	1.3×10^{-10}
100	1.92	8.6×10^{-2}	5.1×10^{-3}	5.7×10^{-1}	5.7×10^{-3}	1.0×10^{-12}	1.3×10^{-9}
1,000	19.21	8.6×10^{-1}	5.1×10^{-2}	5.7×10^{0}	5.7×10^{-2}	1.0×10^{-11}	1.3×10^{-8}
10,000	192.13	8.6×10^{0}	5.1×10^{-1}	5.7×10^{1}	5.7×10^{-1}	1.0×10^{-10}	1.3×10^{-7}

7. Discussion

7.1. Summary of main findings regarding risk of infection

1. In both countries, most of the pathogens investigated pose very low risk to human health from land-applied, conventionally-treated biosolids; that is, annual risk of infection was under the USEPA limit for drinking water, 10^{-4}pppy. At $<10^{-45}$pppy *Campylobacter* infection presented the lowest risk. Higher risk of infection resulted for *Cryptosporidium* and *Ascaris.*

   a. Results suggested care should be taken to assess the risk posed by *Cryptosporidium* oocysts, as the modelled risk was 10^{-5}pppy. However, greater oocyst inactivation in soil in warmer climates (Olson *et al.,* 1999) may reduce risk of infection from this pathogen further below 10^{-4}pppy.

   b. Risk of ascariasis was 10^{-1}pppy, indicating that, where prevalent, this pathogen incurs a concern for public health protection from land-applied biosolids.

2. Risks under worst-case conditions were generally two to three orders of magnitude greater than under typical conditions. However in most cases this still did not pose a significant problem as values were well under 10^{-4}pppy. Risks from viral infection were most sensitive to the worst-case conditions studied.

3. *Ascaris* ova incurred a concerning risk of infection (10^{-1}pppy) in regions of high prevalence. However in regions of low ascariasis prevalence, risk from biosolids reuse to agriculture is very low. Sensitivity analysis revealed that 10,000 ascariasis infections would be required in Victoria to reach the risk of infection this pathogen exhibited for the Mexico data. This implies an incidence of 192 infections per 10^{-5} inhabitants and is therefore highly unlikely for a MEDC context.

4. Results suggest that in regions where *Ascaris* is not endemic, the risk to human health associated with biosolids reuse to agriculture is very low and in many cases negligible (for instance, *Campylobacter*).

7.2. Comparison of Australia and Mexico

7.2.1. Factors affecting pathogen density in biosolids in Australia and Mexico

Two factors influenced pathogen density in wastewater and biosolids; both related to socioeconomic development: pathogen excretion rates and flow of wastewater treated. Pathogen excretion rates were governed by incidence of disease, which was generally lower in Victoria than in either Mexican state. The flow rate of wastewater treated also influenced the concentration of pathogens entering a WWTP. Therefore, a higher disease incidence in one location than another did not necessarily imply higher pathogen concentrations.

For instance, reported incidence of salmonellosis (including typhoid and paratyphoid fever) was higher in Victoria than in DF: 28.70 and 21.14 infections per 10^5 inhabitants, respectively. At $18.38 m^3 s^{-1}$, wastewater treated in Victoria (WSAA *et al.,* 2009; Madden, C., 2010) is more than five times greater than that treated in DF and provides a greater dilution factor for wastewater pathogens. Therefore, concentrations of *Salmonella* in were lower in Victorian wastewater: $6.5x10^3 L^{-1}$ and $7.8x10^4 L^{-1}$ in Victoria and DF, respectively.

7.2.2. Comparison of risk of infection from enteric pathogens in Australia and Mexico

Risk of enteric infection in Mexico was greater than in Victoria by at least one order of magnitude for HAV, *Shigella*, *Salmonella* and *Giardia*. Risk of rotavirus infection in both Victoria and DF was 10^{-12}pppy. Under the conditions established in this model, these risk of infection differences were due to the pathogen loadings in raw wastewater which , with the exception of rotavirus, were all greater in DF than Victoria. (Reported incidence of rotavirus infection in DF and Victoria was 0.03 and 0.5 per 10^5 individuals, respectively; Table 6.2). The results are therefore consistent with the suggestion that incidence of enteric disease is lower in conditions of greater degree of socioeconomic development (Chan, 1997; Jimenez, *et al.*, 2002; Sidhu and Toze, 2009).

Ascaris was the only pathogen in this study which posed an unacceptable risk under both typical and worst-case situations; 10^{-1}pppy is high by the USEPA standard of $<10^{-4}$pppy risk of infection from enteric pathogens in drinking water (Regli *et al.,* 1991).

Ascariasis prevalence is low in MEDCs (Blumenthal, *et al*., 2000; Jimenez *et al*., 2002; Jimenez, 2007) including Australia (EPAV, 2004). Low prevalence of *Ascaris* infection in Victoria highlights the difficulty presented by the EPAV (2004) requirement for a < 2 log *Ascaris* ova removal for a sludge treatment process to qualify as T1-grade.

Furthermore, these treatment standards are not technically justified as ascariasis is not prevalent and the risk posed by other pathogens from agricultural reuse of conventionally-treated biosolids is low.

The results of this investigation suggest that in regions such as Victoria, Australia, where ascariasis incidence is low and biosolids end use is controlled, a multi-barrier approach provides a safe and sustainable strategy to protect human health when biosolids are recycled to agricultural land.

In Mexico, where the risk of *Ascaris* infection is high, a different approach to biosolids management is required in order to provide an effective barrier to *Ascaris* transmission. For unrestricted biosolids use, enhanced treatment methods are required to inactivate *Ascaris* ova. To do so, temperatures in excess of 55°C for over 24 hours can be applied (European Communities, 2001) or pH above 12 (Jimenez *et al.*, 2004). However, sludge treatment is expensive and can be responsible for 40% of the costs of wastewater treatment (UN-HABITAT, 2008) so alternative reuse routes could be explored for areas under economic pressure. Alternatives include industrial processes such as brick-making or land restoration (UN-HABITAT, 2008). Navarro *et al.* (2009) suggested additional measures including crop restrictions to minimize risk of disease and still take advantage of biosolids nutrient value. However, this would only be safe for regulated end uses.

7.3. Comparison of rural and urban Mexico

A large difference between urban and rural water supply and sanitation (WATSAN) provision has been reported in Mexico (WHO/UNICEF, 2000). In 2000, water supply in rural Mexico reached 32% of the population, whereas in urban areas coverage was 87%. Sanitation coverage was 63% and 94% in rural and urban areas, respectively.

Incidence of the enteric diseases included in this investigation was greater in Chiapas than DF (with the exception of HAV, which was equivalent in both). Risk of infection from land application of conventionally-treated biosolids followed the same trend.

As Chiapas is a rural state and DF urban, results suggest the disparity between urban and rural WATSAN coverage may have an impact on the infection rates of enteric disease. Whereas in urban areas there is rising interest in sludge treatment and management due to public health and sustainability reasons, rural areas are more likely to focus their efforts on public health protection through provision of water supply wastewater treatment (UN-HABITAT, 2008)..

As previously discussed, *Ascaris* is the pathogen posing the greatest human health risk in Mexico and enhanced treatment can justify reuse when end use is unrestricted. Jimenez *et al.*, (2004) recommended that in rural areas, where land is readily available and small-scale treatment systems are required, composting is a suitable enhanced biosolids treatment method. Thermophilic anaerobic digestion (TAD) is a more appropriate enhanced treatment method in urban areas where land area is at a premium and skilled labour is available. In remote areas, efforts to provide transmission barriers in the management of excreta at the household level may have the largest impact on health benefits (UN-HABITAT, 2008).

7.4. Model strengths, uses and limitations

7.4.1. Strengths

Modelled bacteria, protozoa (oo)cyst and helminth ova concentrations in wastewater and sludge were comparable to published concentrations, indicating the model successfully integrated pathogen removals reported in the literature for a variety of infectious agents.

The QMRA model presented in this investigation quantitatively followed the path of enteric pathogens from the infected population to the risk of infection through the ingestion of vegetable crops grown in biosolids-amended soil by considering inactivation during conventional wastewater treatment. The resulting risks of infection can be used to provide a scientific basis for recommendations for sludge reuse policy and practice. The microbiological event tree approach followed aids transparency and can be adapted to various situations.

The impact of disease incidence on risk incurred by land-application of biosolids was assessed for data from three different socioeconomic contexts. The man–sludge–crop–man pathway is part of complex system of different treatment processes, sludge management practices and diets – all of which play a part in establishing the risk of infection. The effect of varying disease incidence was studied by establishing several conditions which were equally applied to data for the three regions studied. These included wastewater and sludge treatment trains as well as harvest periods, vegetable handling and consumption. This investigation considers one infection pathway under a variety of situations, and is flexible enough to accommodate conditions changed to suit other contexts.

7.4.2. Applications of the QMRA model

The uses of the QMRA model developed include:

- Use of reported data on incidence of disease to predict pathogen concentrations in biosolids and risk of infection from the agricultural reuse of biosolids.

- Analysis of the impact of different removals on pathogen concentrations in wastewater and biosolids, as well as associated risks of infection.

- If disease incidence data is unavailable, sensitivity analysis can be undertaken to assess risks of infection from the agricultural reuse of biosolids.

7.5. Uncertainties

The most significant uncertainties arising from this investigation include:

7.5.1. Practices governing exposure to pathogens from raw vegetables.

- **Biosolids incorporation into the soil matrix.** Gale (2002) assumed pathogens were homogenously distributed in soil to calculate the probability of contact between a pathogenic particle and the crop surface. In practice, the distribution of pathogens in soil would depend on the biosolids application method used.

- **Raw vegetable consumption.** The assumptions pertaining to vegetable consumption and preparation have a high variation within the community. In QMRA of *Ascaris*

infection from consumption of uncooked spinach and carrots irrigated with raw wastewater, Navarro *et al.* (2009) assumed weekly raw vegetable consumptions of 28-38g and 30-54g respectively. In this investigation, 35g daily raw vegetable intake was assumed, as estimated by Gale (2002) from UK dietary information.

- **Pathogen removal through washing.** It was assumed washing removed one log of pathogenic particles (Gale, 2002; WHO 2006) however this is evidently governed by vegetable handling practices by retailers and consumers, and therefore subject to a large degree of variation.

7.5.2. Pathogen inactivation data

- **Pathogen inactivation in soil.** Experimental pathogen inactivation data ranges were magnified by extrapolation to longer time periods. This had a large impact on the difference between worst-case and typical risks posed by HAV.

  Pathogen decay occurs more slowly under low temperature conditions (Feachem, *et al.* 1983; Strauch *et al.,* 1991). For instance, Olson *et al.,* (1999) found that *Giardia* cysts and *Cryptosporidium* oocysts were undetectable in soil after a 12-week waiting period except at 4°C and -4°C.

7.5.3. Dose-response relationships

- **Pathogens for which dose-response relationships were unavailable.** HAV and *Shigella* dose-response relationships were modelled using rotavirus and *E. coli* O157:H7 dose-response data, respectively. In the case of *Taenia* the conservative assumption one ovum causes infection was adopted.

- **Host response variations**. The dose-response relationships do not necessarily account for variations in host population immunity and therefore do not account for the more sensitive members of the community such as immunocompromised individuals (in the case of *Cryptosporidium* infection) and children under 5 years of age (in the case of rotavirus infection).

- **Use of arithmetic mean to calculate exposure.** Haas (1996) demonstrated mathematically that the arithmetic mean of pathogen exposures can be used in dose response relationships to accurately summarise pathogen exposure data. This may not

apply to all pathogens; Gale (2001) showed that the use of an arithmetic mean for estimation of risk of infection can lead to a threefold overestimate in the case of highly infectious pathogens such as rotavirus.

7.5.4. Pathogen inputs in to raw wastewater

- **Disease under-reporting.** Enteric disease symptoms range from asymptomatic (giardiasis, taeniasis, some salmonelloses, etc) to acute or chronic diarrhoea and brain damage in the case of neurocysticercosis (Cedillo-Rivera *et al.*, 1989; CDC, 2009), it is likely some of the asymptomatic and milder infections will go unreported. The disease will either be left untreated to run its course, or treated through self-medication. In fact, it has been estimated that only 10-20% of incidences of enteric disease are reported (WHO, 1996; Dumontet *et al.,*2001; Gust *et al.*, 2002). This is a factor to be considered with risk of ascariasis, as human infections are often subclinical. However, Ascaris ova are rarely isolated from Victorian sludge (EPAV, 2004), as is the case in other MEDCs (CFR, 1995; Jimenez *et al.*, 2002).

- **Acquired immunity.** Acquired immunity to a pathogen may impact the dose-response relationship, and therefore have an effect on risk estimation.

- **Other sources of pathogens.** Only reported cases of human disease are considered in this investigation. Further pathogen inputs into wastewater may result from animal abattoirs and slurries. However, in spite of these exclusions, pathogen wastewater concentrations predicted by this model were generally consistent with published data**.** Pathogens such as *Salmonella, Campylobacter* and *Taenia* spp. may originate from livestock waste (Hutchinson *et al*., 2004) being washed into wastewater collection systems.

- **Wastewater collection system connectivity**. In this investigation, it was assumed that all excreted pathogens enter the wastewater collection system. While this may well be the case in MEDC regions such as Victoria, LEDCs have a lower degree of connectivity to wastewater collection systems. Therefore, modelled pathogen densities in wastewater (and consequently, in sludge) may overestimate reported values.

8. Conclusions

Disease prevalence data from Australia and Mexico were used to predict pathogen densities in biosolids. Risk of infection from ingestion of raw vegetables grown in conventionally-treated biosolids amended soil was assessed for a selection of bacteria, protozoan (oo)cysts and helminth ova. Risk of infection was calculated using mean and worst-case reported pathogen removals.

1. Under typical conditions, risk of infection from pathogens in conventionally treated biosolids was below the 10^{-4}pppy USEPA limit for drinking water (Regli *et al.,* 1991), except *Ascaris,* which poses a significant risk (10^{-1}pppy) in regions with high incidence of ascariasis.

2. Risk of infection was generally greater in Mexico than in Australia, suggesting risk of infection is greater in LEDCs than in MEDCs, and, in LEDCs, greater in rural areas than in urban areas.

3. In cases where disease incidence data was lacking, sensitivity analysis was carried out revealing that, in order to incur a risk of infection comparable to that in Mexico, 10,000 ascariasis infections would be required in Victoria. The implied incidence is 192 infections per 10^5 inhabitants which is unlikely and suggests *Ascaris* is not a pathogen of concern in Victoria.

4. Adoption of evidence-based microbiological standards for biosolids reuse to agriculture is recommended.

5. The results of this investigation suggest that in regions such as Victoria, Australia, where ascariasis incidence is low and biosolids end use is controlled, a multi-barrier approach provides a safe and sustainable strategy to protect human health when biosolids are recycled to agricultural land.

6. However, enhanced treatment needs to be employed to minimise the risk of ascariasis from land application of biosolids in regions such as Mexico, where disease incidence is high and end use cannot be controlled.

References

ADAS, (2001) *Guidelines for the Application of Sewage Sludge to Agricultural Land.* 3rd Ed. [Online] Available from: www.adas.co.uk/matrix [12th May, 2010]

Amahmid, O., Asmama, S. and Bouhoum, K. (1999) The effect of waste water reuse in irrigation on the contamination level of food crops by *Giardia* cysts and *Ascaris* eggs. *International Journal of Food Microbiology,* 49 (1-2), 19-26.

Andrews. R.J., Lloyd, L.W. and Lerner, D.N. (1998) Sewage sludge disposal to agriculture and other options in the UK. In: Banks, J., Dumpleton, S. and Fermor, M. (eds) *Groundwater Contaminants and their Migration*. Geological Society, London, Special Publications, 128, 63-74.

Arimi, S.M., Fricker, C.R. and Park, R.W.A. (1988) Occurrence of 'thermophilic' *Campylobacter*s in sewage and their removal by treatment processes. *Epidem. Inf.* 101, 279-286.

Asamoa-Bonsu, A. S. (2008) *Assessing the implications of the prevalence of enteric infections in the human population on the microbiological quality of sewage sludge and its treatment for use an agricultural fertiliser.* MSc Environmental Engineering. Imperial College London.

Australian Bureau of Statistics, ABS (2008) *Australian Historical Population Statistics, 2008.* [Online] Available from: http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/3105.0.65.0012008?OpenDocument [Accessed 22nd June, 2010]

Ayed, L.B., Schijiven, J., Alouini, Z., Jemli, M. and Sabbahi, S. (2009) Presence of parasitic protozoa and helminth in sewage and efficiency of sewage treatment in Tunisia. *Parasitological Res.* [Online] 105, 393-406. Available from: DOI: 10.1007/s00436-009-1396-y. [Accessed 15th August, 2010]

Barbier, D., Perrine, D., Duhamel, C., Doublet, R. and Georges, P. (1990) Parasitic hazard with sewage sludge applied to land. *Applied and Environmental Microbiology,* 56 (5), 1420-1422.

Bates, J., Goddard, M.R. and Butler, M. (1984) The detection of rotaviruses in products of wastewater treatment. *J. Hyg. Camb.* 93, 639-643.

Bengtsson, M. and Tillman, A.M. (2004) Actors and interpretations in an environmental controversy: the Swedish debate on sewage sludge use in agriculture. *Resources, Conservation and Recycling.* 42, 65-82.

Berg, G.and Berman, D. (1980) Destruction by Anaerobic Mesophilic and Thermophilic Digestion of Viruses and Indicator Bacteria Indigenous to Domestic Sludges. *Applied and Environmental Microbiology*. 39(2)361-368.

Bitton, Gabriel. (1999) *Wastewater microbiology.* 2nd edition. New York, John Wiley & Sons, Inc.

Blumenthal, U. J., Mara, D. D., Peasey, A., Ruiz-Palacios, G. and Stott, R. (2000) Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. *Bulletin of the World Health Organization.* 78 (9) 1104-1116.

Bowman, D. and Fayer, R. (2005) Chapter 11: Concerns related to protozoan and helminth parasites in biosolids and animal wastes. In: Smith, J.E., Millner, P.D., Jakubowski, W., Goldstein, N. and Rynak, R. (Eds.) *Infectious disease agents in sewage sludge and manure.* p.127-162. The JG Press, Emmaus, PA. [Online] Available from: http://ddr.nal.usda.gov/dspace/bitstream/10113/16828/1/IND43908336.pdf [Accessed 22nd June, 2010]

Bruce, A., Pike, E. B. and Fisher, W. J. (1990) A review of treatment process options to meet the EC sludge directive. *Water and Environment Journal,* 4, 1-13.

Cairncross, S. & Feachem, R. S. (1983) Chapter 1: Engineering and infectious disease, Chapter 12: Composting. In: *Environmental Health Engineering in the Tropics: An Introductory Text.* Chichester, UK, John Wiley & Sons.

Carrington, E.G., Pike, E.B., Auty, D. and Morris, R. (1993) Destruction of faecal bacteria, enteroviruses and ova of parasites in wastewater sludge by aerobic thermophilic and anaerobic mesophilic digestion. [Online] Available from: http://www.cepis.ops-oms.org/muwww/fulltext/repind54/desfae/desfae.html [Accessed on 13th July, 2010]

Cass, J. (2009) *Field and laboratory investigation quantifying the factors responsible for enteric pathogen decay in biosolids-amended agricultural soils.* PhD Thesis. Imperial College London.

CDC (Centre for Disease Control and Prevention) (2009) *DPDx: Laboratory Identification of Parasites of Public Health Concern.* [Online] Available from: http://www.dpd.cdc.gov/dpdx/Default.htm [Accessed 10th July, 2010].

CDPCU (Communicable Disease Prevention and Control Unit) (2010) *Infectious diseases epidemiology and surveillance*. [Online] Available from: http://www.health.vic.gov.au/ideas [Accessed 1st July, 2010].

CEC (Council of European Communities) (1991) Council Directive 1999/31/EC of 26 April 1999 on the landfill of waste. [Online] Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31999L0031:EN:HTML [Accessed 23rd July, 2010]

CEC (Council of European Communities) (1991) Council Directive 91/271/EEC of 21 May 1991 concerning urban waste-water treatment [Online] Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31991L0271:EN:NOT [Accessed 23rd July, 2010]

CFR (Code of Federal Regulations) (1995) *Standards for the use or disposal of sewage sludge.* Title 40. Part 503. [Online]. Available from: http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=%2Findex.tpl

Cedillo-Rivera, R., Enciso-Moreno, J.A., Martinez-Palomo, A. and Ortega-Pierres, G. (1989) *Giardia lamblia*: isoenzyme analysis of 19 axenic strains isolated from symptomatic and asymptomatic patients in Mexico. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 83, 644-646.

Chan, M-S. (1997) The global burden of intestinal nematode infections — Fifty years on. *Parasitology Today,* 13 (11), 438-443.

Chauret, C., Springthorpe, S. and Sattar, S. (1999) Fate of *Cryptosporidium* oocysts, *Giardia* cysts, and microbial indicators during wastewater treatment and anaerobic sludge digestion. *Canadian Journal of Microbiology,* 45, 257-262.

Coleman, M. E. and Marks, H. M. (2000) Mechanistic modelling of Salmonellosis. *Quantitative Microbiology,* 2 (3), 227-248.

CONAGUA (Comisión Nacional del Agua; National Water Commission, Mexico) (2010) *Estadísticas del Agua* (Water Statistics) Mexico [Online] Available from: www.conagua.gob.mx [28th June, 2010]

Cooper and Olivieri, (1998) Infectious disease concerns in wastewater reuse. In: Asano, T. (Ed.) *Wastewater reclamation and reuse.* Lancaster, Technomic Publishing.

Copeland, C. (1999) *Ocean Dumping Act A summary of the law.* CRS Report for Congress. [Online] Available from: http://ncseonline.org/nle/crsreports/marine/mar-25.cfm

Crockett, C.S., Haas, C.N, Fazil,A., Rose, J.B. and Gerba C.P. (1996) Prevalence of shigellosis in the US: Consistency with dose-response information. *Int. J. Food. Microbiol.* 30(1-2)87-99.

Dahab , M.F. and Surampalli, R.Y. (2002) Effects of aerobic and anaerobic digestion systems on pathogen and pathogen indicator reduction in municipal sludge. *Water Science and Technology*. 46 (10) 181-187.

Dale, D. C. and Mata, L. J. (1968) Intestinal Bacterial Flora in Malnourished Children with Shigellosis. *American Journal of Tropical Medicine and Hygiene,* 17 (3), 397-403.

Defra (Department of Environment, Food and Rural Affairs). (2006) *e-Digest Statistics about: Waste and Recycling. Sewage sludge* [Online] Available from: http://www.defra.gov.uk/evidence/statistics/environment/waste/wrsewage.htm#wrtb11 [1st August, 2010]

Defra (Department of Environment, Food and Rural Affairs). (2007) *Waste Strategy for England. Annexes.* [Online] Available from http://www.defra.gov.uk/environment/waste/strategy/strategy07/documents/waste07-annexes-all.pdf [1st August, 2010]

Dumontet, S., Scopa, A., Kerje, S. and Krovacek, K. (2001) The importance of pathogenic organisms in sewage and sewage sludge. *Journal of the Air & Waste Management Association,* 51 (6), 848-860.

DuPont, H. L., Chappell, C. L., Sterling, C. R., Okhuysen, P. C., Rose, J. B. and Jakubowski, W. (1995) The Infectivity of *Cryptosporidium* parvum in Healthy Volunteers. *New England Journal of Medicine,* 332 (13), 855-859.

Ensink, J., Mahmood, T. And Dalsgaard, A. (2007) Wastewater-irrigated vegetables: market handling versus irrigation water quality. *Tropical Medicine and International Health* [Online] 12, 2-7. Available from: DOI: 10.1111/j.1365-3156.2007.01935.x. [Accessed 2nd July, 2010]

EPAV (Environmental Protection Agency, Victoria) (2004) *Guidelilnes for Environmental Management: Biosolids Land Application.*

European Communities (2001) *Evaluation of sludge treatments for pathogen reduction – Final report.* For the European Commission Directorate-General Environment. Study Contract No. B4-3040/2001/322179/MAR/A2. [Online] Available from: http://ec.europa.eu/environment/waste/sludge/pdf/sludge_eval.pdf. [Accessed 29th August, 2010]

FAO (Food and Agriculture Organization of the United Nations) / WHO (World Health Organization) (2000) *Joint FAO/WHO Activities on Risk Assessment of Microbiological Hazards in Foods Risk Assessment: Salmonella spp. in Broilers and Eggs.* Report No. MRA 00/03

Fazil, A.M. (2005) *A primer on risk assessment modelling: focus on seafood products*. FAO Fisheries Technical Paper T462 [Online] Available from: http://www.fao.org/docrep/009/a0238e/A0238E04.htm [Accessed 29th September, 2010]

Feachem, R. G., Bradley, D. J. & Garelick, H. (1983) *Sanitation and disease: health aspects of excreta and waste management.* New York, Wiley.

Gaasenbeek, C.P.H. and Borgsteede, F.H.M. (1998) Studies on the survival of *Ascaris suum* eggs under laboratory and simulated field conditions. *Veterinary Parasitology* 75(2-3)227-234.

Gale, P. (2003) Using event trees to quantify pathogen levels on root crops from land application of treated sewage sludge. *Journal of Applied Microbiology,* 94, 35-47.

Gale, P. (2002) *Pathogens in biosolids - Microbiological Risk.* UK Water Industry Research Ltd. Environment Agency R&D Technical Report Ref. No. P2-161 (Phase III).

Gale, P. (2001) Developments in microbiological risk assessment for drinking water. *Journal of Applied Microbiology.* 91, 191-205.

Gantzer, C., Gaspard, P., Galvez, L., Huyard, A., Dumouthier, N. and Schwartzbrod, J. (2001) Monitoring of bacterial and parasitological contamination during various treatment of sludge. *Water Research*. 35(16)3763-3770

Gaspard, P., Wiart, J. and Schwartzbrod, J.. (1997) Parasitological Contamination Of Urban Sludge Used For Agricultural Purposes. *Waste Management & Research,* 15 (4), 429-436.

Gavaghan, P.D., Sykora, J.L., Jakubowski, W., Sorber, C.A., Sninsky, A.M., Lichte, M.D. and Keleti, G. (1993) Inactivation of *Giardia* by anaerobic digestion of sludge. *Water Science and Technology*. 27(3-4)111-114.

Geldreich, E.E., Nash, H.D., Reasoner, D.J. and Taylor, R.H. (1972) The necessity of controlling bacterial populations in potable waters: community water supply. *Journal of the American Water Works Association.* 64(9)596-602.

Gerba, C. P., Castro-del Campo, N., Brooks, J. P. and Pepper, I. L. (2008) Exposure and risk assessment of *Salmonella* in recycled residuals. *Water Science and Technology,* 57 (7), 1061-1065.

Gerba, C.P., Pepper, I.L. and Whitehead, L.F. (2002) A risk assessment of emerging pathogens of concern in the land application of biosolids. *Water Science and Technology*. 46(10)225-230.

Gerba, C. P., Rose, J. B. and Haas, C. N. (1996) Waterborne rotavirus: a risk assessment. *Water Research,* 30 (12), 2929-2940.

Godfree, A. and Farrell, J. (2005) Processes for managing pathogens. *Journal of Environmental Quality,* 34 (1), 105-13.
Goulding K (2008) Updating RB209: Defra's new 'Fertiliser Manual'. Presented at Bioresources to Land: Managing Nutrients to Complete the Cycle. Sustainable Organic Resources Partnership. 15 October, Royal Academy of Engineering, London.
Graczyk, T. K., Kacprzak, M., Neczaj, E., Tamang, L., Graczyk, H., Lucy, F. E. and Girouard, A. S. (2008) Occurrence of *Cryptosporidium* and *Giardia* in sewage sludge and solid waste landfill leachate and quantitative comparative analysis of sanitization treatments on pathogen inactivation. *Journal of Environmental Research,* 106, 27-33.
Gust, Ian D. (1992) Epidemiological patterns of hepatitis A in different parts of the world. *Vaccine,* 10 (Supplement 1), S56-S58.
Haas, C. L., Rose, J. B. and Gerba, C. P. (1999) *Quantitative microbial risk assessment.* New York, John Wiley & Sons Inc.
Haas, C. L. (1996) How to average microbial densities to characterize risk. *Water Research*. 3, 1,036-1,038.
Haas, C.L., Crockett, C.S., Rose, J.B., Gerba, C.P. and Fazil, A.M. (1996) Assessing the risk posed by oocysts in drinking water. *Journal of American Water Works Association* 88(9)131-136.
Haas, C.L., Rose, J.B., Gerba, C. and Regli, S. (1993) Risk assessment of virus in drinking water. *Risk Analysis.* [Online] 13(5)545-552. Available from: http://74.125.155.132/scholar?q=cache:YuppTRTQuJoJ:scholar.google.com/&hl=en&as_sdt=2000 [Accessed 23rd August, 2010]
HMSO (Her Majesty's Stationery Office) (1996) *Royal Commission on Environmental Pollution Nineteenth Report: Sustainable Use of Soil.* London, HMSO.
Horak, P. (1992) Helminth eggs in the sludge from three sewage treatment plants in Czechoslovakia. *Folia Parasitologica*. 39, 153-157.
Horan, N. J., Fletcher, L., Betmal, S. M., Wilks, S. A. and Keevil, C. W. (2004) Die-off of enteric bacterial pathogens during mesophilic anaerobic digestion. *Water Research,* 38 (5), 1113-1120.
Horan, N. and Lowe, P. (2001) *Pathogens in biosolids – The fate of pathogens in sewage treatment.* UK Water Industry Research Ltd. Environment Agency R&D Technical Report Ref. No. P2-161 (Phase II).
Hu, C. J., Gibbs, R. A., Mort, N. R., Hofstede, H. T., Ho, G. E. and Unkovich, I. (1996) *Giardia* and its implications for sludge disposal. *Water Science and Technology,* 34 (7-8), 179-186.
Hurst, C. J., Gerba, P. G. and Cech, I. (1980) Effects of Environmental Variables and Soil Characteristics on Virus Survival in Soil. *Applied and Environmental Microbiology,* 40 (6), 1067-1079.
Hutchinson, M.L., Walters, L.D., Moore, A., Crookes, K.M. and Avery, S.M. (2004) Effect of Length of Time before Incorporation on Survival of Pathogenic Bacteria Present in Livestock Wastes Applied to Agricultural Soil. *Applied and Environmental Microbiology*. [Online] 70(9)5111-5118. Available from DOI: 10.1128/AEM.70.9.5111–5118.2004 [5th August, 2010]
INEGI (Instituto Nacional de Estadistica y Geografia & National Institute of Statistics and Geography). (2005) *Conteo de Poblacion y Vivienda (Population and Dwelling Count).* [Online] Available from: http://www.inegi.org.mx/sistemas/olap/proyectos/bd/consulta.asp?p=10215&c=16851&s=est [Accessed 8th June, 2010].
Jakubowski, W. (1984) Detection of *Giardia* cysts in drinking water: state-of-the-art. In: Stanley, L., Erlandsen, E. & Meyer, A. (eds.) *Giardia and giardiasis:biology, pathogenesis and epidemiology.* New York, Plenum press.
Jenkins, M.B., Bowman, D.D. and Ghiorse, W.C. (1998) Inactivation of *Cryptosporidium parvum* oocysts by ammonia. *Applied and Environmental Microbiology*. 64(2)784-788.
Jimenez, B. (2007) Helminths (Worms) Eggs Control in Wastewater and Sludge. [Online] In: *UNESCO International Symposium on New Directions in Urban Water Management.* Paris, France. 12-14th September, 2007. Available from: http://www2.gtz.de/Dokumente/oe44/ecosan/en-helminths-eggs-control-wastewater-sludge-2007.pdf [Accessed 12th January, 2010].

Jimenez, B., Barrios, J.A., Mendez, J.M. and Diaz, J. (2004) Sustainable sludge management in developing countries. *Water Science and Technology*. 49(10)251-258.

Jimenez, B., Maya, C., Sanchez, E., Romero, A., Lira, L. and Barrios, J. A. (2002) Comparison of the quantity and quality of the microbiological content of sludge in countries with low and high content of pathogens. *Water Science and Technology,* 46 (10), 17-24.

Jimenez, B., Chavez, C. M. and Jardines, L. (2001) Removal of microorganisms in different stages of wastewater treatment for Mexico City. *Water Science and Technology,* 43 (10), 155-162.

Jimenez, B., Chavez, A., Leyva, A. and Tchobanoglous, G. (2000) Sand and synthetic medium filtration of advanced primary treatment effluent from Mexico City. *Water Research,* 34 (2), 473-480.

Jimenez, B. and Chavez, A. (1998) Removal of Helminth Eggs in an Advanced Primary Treatment with Sludge Blanket. *Environmental Technology.* [Online] 19(11) 1061-1071. Available from: DOI: 10.1128/AEM.70.9.5111–5118.2004. [Accessed on 10th July, 2010]

Jones, K., Betaieb, M. and Telford, D.R. (1990) Seasonla variation of thermophilic *Campylobacter*s in sewage sludge. *Journal of Applied Bacteriology*. 69, 185-189.

Kearney, T.E., Larkin, M.J., Frost, J.P. and Levett, P.N. (1993) Survival of pathogenic bacteria during mesophilic anaerobic digestion of animal waste. *Journal of Applied Bacteriology.* 75, 215-219.

Koenraad, P.M.F.J., Rombouts, F.M. and Notermans, S.H.W. (1997) Epidemiological aspects of thermophilic *Campylobacter* in water-related environments: A review. *Water Environment Research.* 69(1)52-63.

Laffite-Trouque, S. and Forster, C.F. (2002) The use of ultrasound and γ-irradiation as pre-treatments for the anaerobic digestion of waste activated sludge at mesophilic and thermophilic temperatures. *Bioresource Technology*. 84 (2002) 113-118.

Lang, N.L. and Smith, S.R. (2008) Time and temperature inactivation kinetics of enteric bacteria relevant to sewage sludge treatment processes for agricultural use. *Water Research.* 42, 2229-2241.

Lasobras, J., Dellunde, J., Jofre, J. and Lucena, F. (1999) Occurrence and levels of phages proposed as surrogate indicators of enteric viruses in different types of sludges. *Journal of Applied Microbiology*. 86, 723-729.

Li-Ng, J.J. (Secretaría de Salud) Email correspondence with Elisa Jimenez Grant 27th July, 2010).

Lucena, F., Duran, A.E., Morón, A., Calderón, E., Campos, C., Gantzer, C. and Skraber, S. (2004) Reduction of bacterial indicators and bacteriophages infecting faecal bacteria in primary and secondary wastewater treatments. *Journal of Applied Microbiology* [Online] 97, 1069-1076. Available from: DOI: 10.1111/j.1365-2672.2004.02397.x [6th August, 2010]

Lucero-Ramirez, B. (2000) *The effects of time and temperature on the fate of pathogens and indicator bacteria during municipal wastewater sludge – mesophilic anaerobic digestion, air-drying, and composting.* PhD thesis. University of Texas at Austin. [Online] Available from: http://www.crwr.utexas.edu/reports/pdf/2000/rpt00-14.pdf [Accessed 14th July, 2010]

Madden, C. (South East Water) Email correspondence with Elisa Jimenez Grant 12th July, 2010.

Mara, D. and Horan, N. (eds) (2003) Handbook of water and wastewater microbiology. London, Academic Press.

Mara, D. D. and Horan, N. J. (2002) Sludge to Land: Microbiological Double Standards. *Water and Environment Journal,* 16, 249-252.

Mayer, C.L. and Palmer, C.J. (1996) Evaluation of PCR, Nested PCR, and Fluorescent Antibodies for Detection of *Giardia* and *Cryptosporidium* Species in Wastewater. *Applied and Environmental Microbiology*. 62(6)2081-2085.

Medema, G. J. and Schijven, J. F. (2001) Modelling the sewage discharge and dispersion of *Cryptosporidium* and *Giardia* in surface water. *Water Research,* 35 (18), 4307-4316.

Metcalf and Eddy Inc (2004) *Wastewater Engineering: Treatment and Reuse*. 4th Ed. International Edition. Boston, McGraw Hill Higher Education.

NWC (National Water Commission). (2005) *Australian Bureau of Statistics water accounts.* [Online] Available from: http://www.water.gov.au/WaterUse/AustralianBureauofStatisticswateraccounts/index.aspx?Menu=Level1_4_1 [Accessed on 4th June, 2010]

Navarro, I., Teunis, P. F. M., Moe, C. and Jimenez, B. *Approaches to Evaluate and Develop Health Risk-Based Standards Using Available Data.* [Online] Available from: http://www.idrc.ca/en/ev-151710-201-1-DO_TOPIC.html [Accessed 30th July 2010].

Nelson, K. L. (2003) Concentrations and inactivation of *Ascaris* eggs and pathogen indicator organisms in wastewater stabilization pond sludge. *Water Science and Technology,* 48 (2), 89-95.

Newton, W. L., Bennet, J. B. and Figgat, W. B. (1948) Observations on the effects of various sewage treatment processes upon eggs of *Taenia* saginata. *American Journal of Hygiene,* 49, 166-175.

Olson, M.E., Goh, J., Phillips, M., Guselle, N. and McAllister, T.A. (1999) *Giardia* cyst and *Cryptosporidium* oocyst survival in water, soil and cattle feces. *J. Environ. Qual.* 28, 1991-1996.

OzFoodNet Network. (2009) *Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: Annual report of the OzFood Net Network, 2007.* [Online] Available from: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3204c.htm [Accessed 7th June, 2010].

Payment, P., Plante, R. and Ceika, P. (2001) Removal of indicator bacteria, human enteric viruses, *Giardia* cysts, and *Cryptosporidium* oocysts at a large wastewater primary treatment facility. *Canadian Journal of Microbiology,* 47, 183-193.

Pepper, I. L., Zerzghi, H., Brooks, J. P. and Gerba, C. P. (2008) Sustainability of Land Application of Class B Biosolids. 37, S-58-S-67.

Pepper, I. L., Brooks, J. P. and Gerba, C. P. (2006) Pathogens in Biosolids. In: Sparks, Donald L. (ed.) *Advances in Agronomy.* Academic Press, pp. 1-41.

Pederson, D.C. (1981) *Project Summary: Density Levels of Pathogenic Organisms in Municipal Wastewater Sludge – A Literature Review*. United States Environmental Protection Agency. EPA-600/S2-81-170

Pettersen, S.A. and Ashbolt, N.J. (2003) *WHO Guidelines for the Safe Use of Wastewater and Excreta in Agriculture: Microbial Risk Assessment Section* [Online] Available from: http://www.who.int/water_sanitation_health/wastewater/mrareview.pdf [15th June, 2010]

Pike, E. (1986) *Infectivity of Taenia eggs after sludge treatment*. Final report to the Department of the Environment B(ME9147 SLD)

Powell, M.R., Ebel,E., Schlosser, W., Walderhaug, M. and Kause, J. (2000) Dose-response for Escherichia coli O157:H7. *Quantitative microbiology.* 2(2)121-163

PSD (Pollution Solutions and Designs) (2009) *Review of Biosolids Guidelines: Summary*. Australian and New Zealand Biosolids Partnership. Australia.

Rao, V. C., Metcalf, T. G. and Melnick, J. L. (1986) Human viruses in sediments, sludges and soils. *Bulletin of the World Health Organisation,* 64 (1), 1-14.

Regli S., Rose, J.B. and Haas, C.N. (1991) Modeling the risk from *Giardia* and viruses in drinking water. *Journal of the American Water Works Association*, 83(11)76-84.

Reimers, R.S., Little, M.D., Englande, A.J., McDonell, D.B., Bowman, D.D. and Hughes, J.M. (1986) *Project Summary: Investigation of Parasites in Sludges and Disinfection Techniques*. United States Environmental Protection Agency. EPA/600/S1-85/022.

Rigby, H., Pritchard, D., Collins, D., Walton, K., Allen, D. and Penney, N. (2010) Improving guidelines for the plant available nitrogen value of biosolids from wastewater treatment. *Journal of residuals science and technology* 7(1)13-19

Robertson, L.J., Forberg, T., Hermansen, L., Gjerde, B.K., Alvsvag, J.O. and Langeland, N. (2006) *Cryptosporidium parvum* infections in Bergen, Norway, during an externsive outbreak of waterborne giardiasis in autumn and winter 2004. *Applied and Environmental Microbiology*. 72(3)2218-2220.

Robertson, L.J., Paton, C.A., Campbell, A.T., Smith, P.G., Jackson, M.H., Gilmour, R.A., Black, S.E., Stevenson, D.A. and Smith, H.V. (2000) *Giardia* cysts and *Cryptosporidium* oocysts at sewage treatment works in Scotland, UK. *Wat. Res.* 34(8)2310-2322.

Rose, J. B., Gerba, C. P. and Jakubowski, W. (1991) Survey of potable water supplies for *Cryptosporidium* and *Giardia*. *Environmental Science and Technology,* 25 (8), 1393-1400.

Schwartzbrod, J. and Banas, S. (2003) Parasite contamination of liquid sludge from urban wastewater treatment plants. *Water Science and Technology.* 47(3)163-166.

Secretaria de Salud (Mexican Ministry of Health). (2008) SUIVE: *Anuarios de morbilidad (Morbidity annual compilation 1984 - 2008).* [Online] Available from: http://www.dgepi.salud.gob.mx/anuario/html/anuarios.html [Accessed 12th July, 2010].

SEMARNAT (Secretaria de Medio Ambiente y Recursos Naturales, Ministry of Environment and Natural Resources) (2003) *Protección ambiental.- Lodos y biosólidos.-Especificaciones y límites máximos permisibles de contaminantes para su aprovechamiento y disposición final. (*Environmental protection. Sludges and biosolids. Specifications and maximum permissible limits for contaminants for their best utilization and final disposition). NOM-004-SEMARNAT-2002. Mexico.

Sidhu, J.P.S. and Toze, S.G. (2009) Human pathogens and their indicators in biosolids: A literature review. *Environment International* 35, 187-201.

Sludge (Use in Agriculture) Regulations 1989. (SI 1989/1263)

Smith, S. R., Lang, N. L., Cheung, K. H. M. and Spanoudaki, K. (2005) Factors controlling pathogen destruction during anaerobic digestion of biowastes. *Journal of Waste Management,* 25, 417-425.

Smith, S.R. (2007) Recycling biosolids to land. In: Hislop, H. (Ed.) *The nutrient cycle: closing the loop.* Green Alliance. p.19-25. [Online] Available from: http://www.green-alliance.org.uk/uploadedfiles/Publications/reports/TheNutrientCycle.pdf [Accessed 23rd July, 2010]

Smith, S.R. (2009) Organic contaminants in sewage sludge (biosolids) and their significance for agricultural recycling. *Phil. Trans. R. Soc. A.* [Online] Available from: doi: 10.1098/rsta.2009.0154. [Accessed: 26th August, 2010]

Sobsey, M.D., Hall, R.M. and Hazard, R.L. (1995) Comparative reductions of hepatitis A virus, enteroviruses and coliphage MS2 in miniature soil columns. *Water Science and Technology*. 31(5-6)203-209

Spears, A. (2010) (Australian Water Association). Personal Communication.

Spillman, S.K., Traub, F., Schwyzer, M. and Wyler, R. (1987) Inactivation of Animal Viruses during Sewage Sludge Treatment. *Applied and Environmental Microbiology*. 53(9) 2077-2081.

Sommers, L. E. (1977) Chemical Composition of Sewage Sludges and Analysis of Their Potential Use as Fertilizers. *Journal of Environmental Quality,* 6 (2), 225-232.

Sterritt, R. M. & Lester, J. N. (1988) *Microbiology for Environmental and Public Health Engineers.* London, E & F.N. Spon.

Storey, G.W. (1987) Survival of tapeworm eggs, free and in proglottids, during simulated sewage treatment processes. *Water Research*. 21(2) 199-203.

Straub, T.M., Pepper, I.L. and Gerba, C.P. (1993) Hazards from pathogenic microorganisms in land-disposed sewage sludge. *Reviews of Environmental Contamination and Toxicology*. 132, 55-91.

Strauch, D. (1991) Survival of pathogenic micro-organisms and parasites in excreta, manure and sewage sludge. *Rev. Sci. Tech. Off. Int. Epiz.* 10(3)813-846.

Taylor, David N., Perlman, Daniel M., Echeverria and Peter D., Lexomboon, Udom & Blaser, Martin J. (1993) *Campylobacter* Immunity and Quantitative Excretion Rates in Thai Children. *The Journal of Infectious Diseases,* 168 (3), 754-758.

Thomaz-Soccol, V., Paulino, R.C., Castro, E.A. and Andreoli, C.V. (1997) Helminth eggs viability in sewage sludge and biosolids sludge in Curitiba, Paraná, Brazil. *Braz. Arch. Boil. Technol.* 40(4)829-836

Tierney, J.T., Sullivan, R. and Larkin, E.P. (1977) Persistence of Poliovirus 1 in Soil and on Vegetables Grown in Soil Previously Flooded with Inoculated Sewage Sludge or Effluent. *Applied and Environmental Microbiology*. 33(1) 109-113

Toze, S. (1997) *Microbial Pathogens in Wastewater: Literature Review for Urban Water Systems Multi-divisional Research Program*. CSI RO Land and Water. Technical Report No. 1/97

Traub, F., Spillman, S.K and Wyler, R. (1986) Method for Determining Virus Inactivation during Sludge Treatment Processes. *Applied and Environmental Microbiology*. 52(3) 498-503.

UN-HABITAT (United Nations Human Settlements Programme) LeBlanc, R.J., Matthews, P. and Richard, P.R. (Eds.) (2008) *Global Atlas of excreta, wastewater sludge and biosolids management: moving forward the sustainable and welcome uses of a global resource.* UN-HABITAT. Kenya.

USEPA (United States Environmental Protection Agency) (2003) *Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge*. [Online] Report No. EPA/625/R-92/013. Available from: http://www.epa.gov/nrmrl/pubs/625r92013/625r92013.htm [Accessed 25th June, 2010]

Villacorta-Martinez de Matutana, I., Ares-Mazás, M.E., Duran-Oreiro, D. and Lorenzo-Lorenzo, M.J. (1992) Efficacy of activated sludge in removing *Cryptosporidium parvum* oocysts from sewage. *Applied and Environmental Microbiology*. 58(11)3514-35-16.

Wang, M. (1997) Land application of sewage sludge in China. *The Science of the Total Environment,* 197, 149-160.

Ward, R. L., Bernstein, D. I., Young, E. C., Sherwood, J. R., Knowlton, D. R. and Schiff, G. M. (1986) Human Rotavirus Studies in Volunteers: Determination of Infectious Dose and Serological Response to Infection. *The Journal of Infectious Diseases,* 154 (5), 871-880.

WaterUK (2006) *Wastewater treatment and recycling.* [Online] Available from: http://www.water.org.uk/home/news/archive/wastewater/wastewater-treatment-11-05-2006 [Accessed 22nd July, 2010]

WSAA (Water Services Association of Australia), NWC (National Water Commission) & NWI (National Water Initiative) parties (Australia). (2009) *National Performance Report 2007 - 2008: Urban Water Utilities.* Australia, .

Watson, D.C. (1980) The survival of *Salmonella*e in sewage sludge applied to arable land. *Water Pollution Control.* 79(1)11-18.

Wen, Q.; Tutuka, C., Keegan, A. and Jin, B.. (2009) Fate of pathogenic microorganisms and indicators in secondary activated sludge wastewater treatment plants. *Journal of Environmental Management,* 90 (3), 1442-1447.

Westrell, T., Schonning, C., Stenstrom, T. A. and Ashbolt, N. J. (2004) QMRA (quantitative microbial risk assessment) and HACCP (hazard analysis and critical control points) for management of pathogens in wastewater and sewage sludge treatment and reuse. *Water Science and Technology,* 50 (2), 23-30.

Williams, P.T. (2005) *Waste Treatment and Disposal.*2nd Ed. Great Britain, John Wiley & Sons, Ltd.

WHO (World Health Organization) (2010) *Taeniasis/Cysticercosis*. [Online] Available from: http://www.who.int/zoonoses/diseases/*Taenia*sis/en/ [Accessed 18th June, 2010]

WHO (World Health Organization). (2007) *International statistical classification of diseases and related health problems, 10th revision.* [Online] Available from: http://apps.who.int/classifications/apps/icd/icd10online/ [Accessed 18th June, 2010].

WHO (World Health Organization) (2006) *Guidelines for safe use of wastewater, excreta and greywater*. [Online] Available from: http://www.who.int/water_sanitation_health/wastewater/gsuww/en/index.html [Accessed 20th February, 2010]

WHO/UNICEF (2000) *Global Water Supply and Sanitation Assessment 2000 Report* [Online] Available from: http://www.who.int/docstore/water_sanitation_health/Globassessment/GlobalTOC.htm [Accessed 4th July, 2010]

Yates, M.V. and Gerba, C.P. (1998) *Microbial considerations in wastewater reclamation and reuse.* In: Asano, T. (ed.), Wastewater Reclamation and Reuse, vol. 10 Water Quality Management Library. Technomic Publishing Co. Inc., Lancaster PA, p. 437-488.

Yaziz, M.I. and Lloyd, B.J. (1979) The removal of *Salmonella*s in conventional sewage treatment processes. *Journal of Applied Biotechnology*. 46, 131-142.

Zábranská, J., Dohányos, M., Jeníček, P., Růžičiková, H. and Vránová, A. (2003) Efficiency of autothermal thermophilic aerobic digestion and themophilic anaerobic digeswtion of municipal wastewater sludge in removing *Salmonella* spp. and indicator bacteria. *Water Science and Technology*. 47(3)151-156.

Zaleski, K.J., Josephson, K.L., Gerba, C.P. and Pepper, I.L. (2005) Potential Regrowth and Recolonization of *Salmonella*e and Indicators in Biosolids and Biosolid-Amended Soil. *Applied and Environmental Microbiology*. [Online] 71(7) 3701-3708. Available from: DOI: 10.1128/AEM.71.7.3701–3708.2005 [Accessed 4th August, 2010]

Appendices

Appendix A. Population density and incidence of Ascariasis in Mexico

(INEGI, 2005; Secretaria de Salud, 2008)

State	Area / km^{-2}	Population	Population density / inhabitants km^{-2}
Distrito Federal	1,479	8,720,916	5,896.5
México	21,355	14,007,495	655.9
Chihuahua	5,191	3,241,444	624.4
Morelos	4,950	1,612,899	325.8
Tlaxcala	4,016	1,068,207	266.0
Aguascalientes	5,471	1,065,416	194.7
Guanajuato	30,491	4,893,812	160.5
Puebla	33,902	5,383,133	158.8
Querétaro Arteaga	11,499	1,598,139	139.0
Hidalgo	20,813	2,345,514	112.7
Veracruz de Ignacio de la Llave	71,699	7,110,214	99.2
Jalisco	80,386	6,752,113	84.0
Tabasco	25,267	1,989,969	78.8
Michoacán de Ocampo	59,928	3,966,073	66.2
Nuevo León	64,924	4,199,292	64.7
Guerrero	64,281	3,115,202	48.5
Yucatán	38,402	1,818,948	47.4
Sinaloa	58,328	2,608,442	44.7
Baja California	69,921	2,844,469	40.7
San Luis Potosí	63,068	2,410,414	38.2
Tamaulipas	79,384	3,024,238	38.1
Oaxaca	93,952	3,506,821	37.3
Nayarit	26,979	949,684	35.2
Coahuila de Zaragoza	74,211	2,495,200	33.6
Chiapas	149,982	4,293,459	28.6
Quintana Roo	50,212	1,135,309	22.6
Zacatecas	73,252	1,367,692	18.7
Campeche	50,812	754,730	14.9
Sonora	182,052	2,394,861	13.2
Durango	123,181	1,509,117	12.3
Baja California Sur	73,475	512,170	7.0
Colima	247,938	567,996	2.3

Appendix B. HAV risk assessment

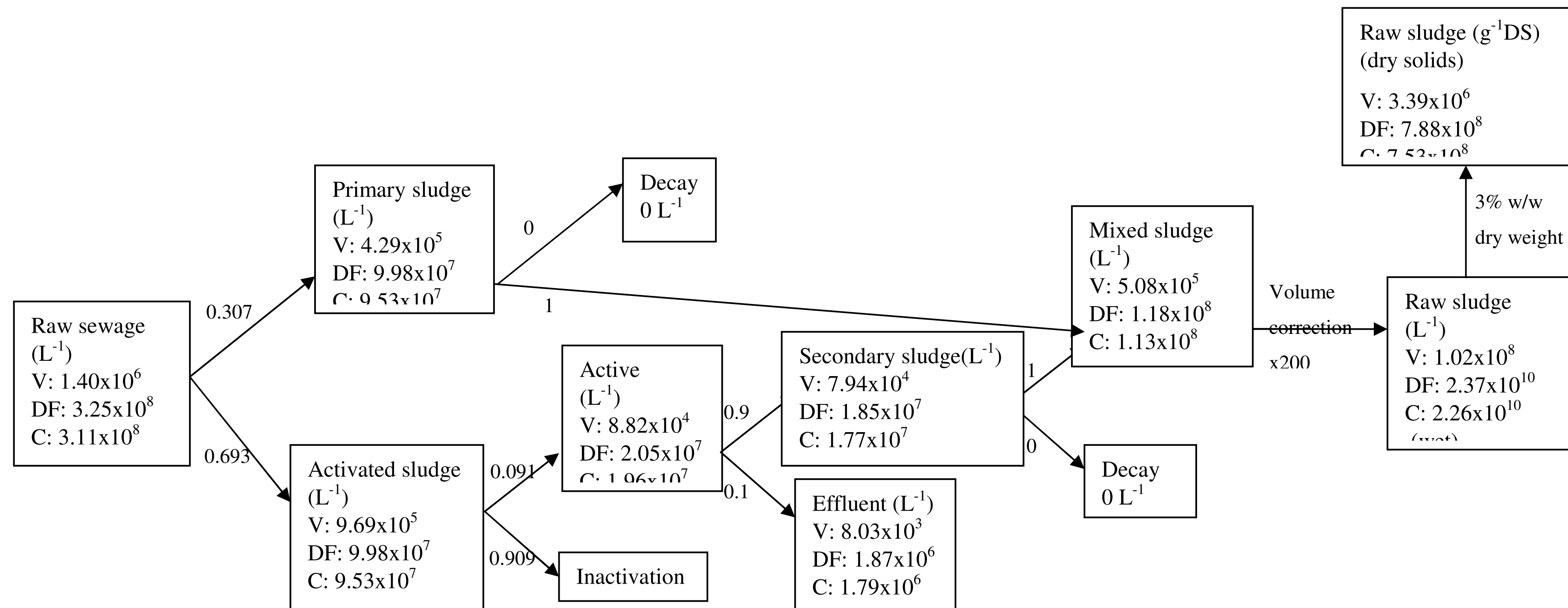


Figure B.1. Microbiological event tree for HAV transfer and decay from raw wastewater to raw sewage sludge (V – Victoria, DF – Distrito Federal, Mexico; C – Chiapas, Mexico)

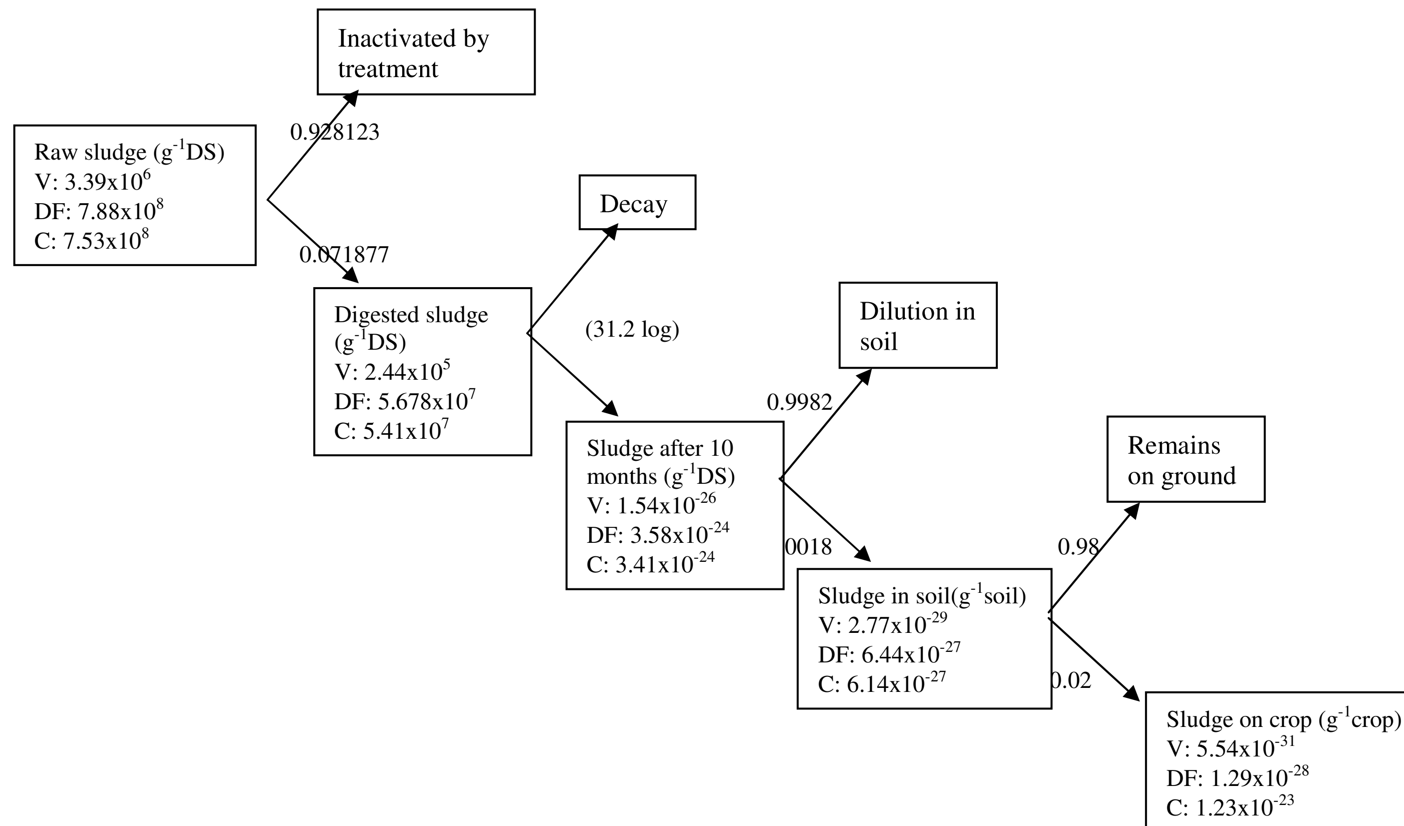


Figure B.2. Microbiological event tree for HAV transfer and decay from raw sewage sludge to sludge crop loading (V – Victoria, DF – Distrito Federal, Mexico; C – Chiapas, Mexico)

Appendix C. Rotavirus risk assessment

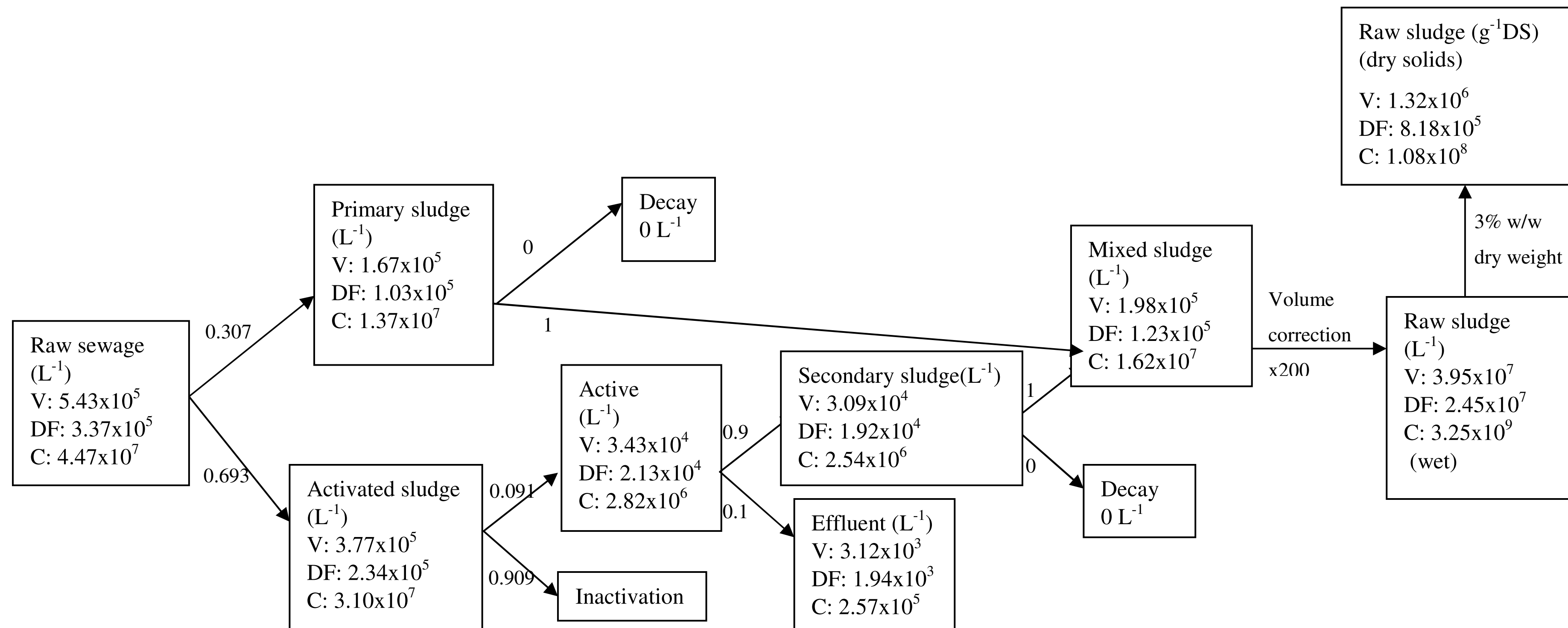


Figure C.1. Microbiological event tree for rotavirus transfer and decay from raw wastewater to raw sewage sludge (V – Victoria, DF – Distrito Federal, Mexico; C – Chiapas, Mexico)

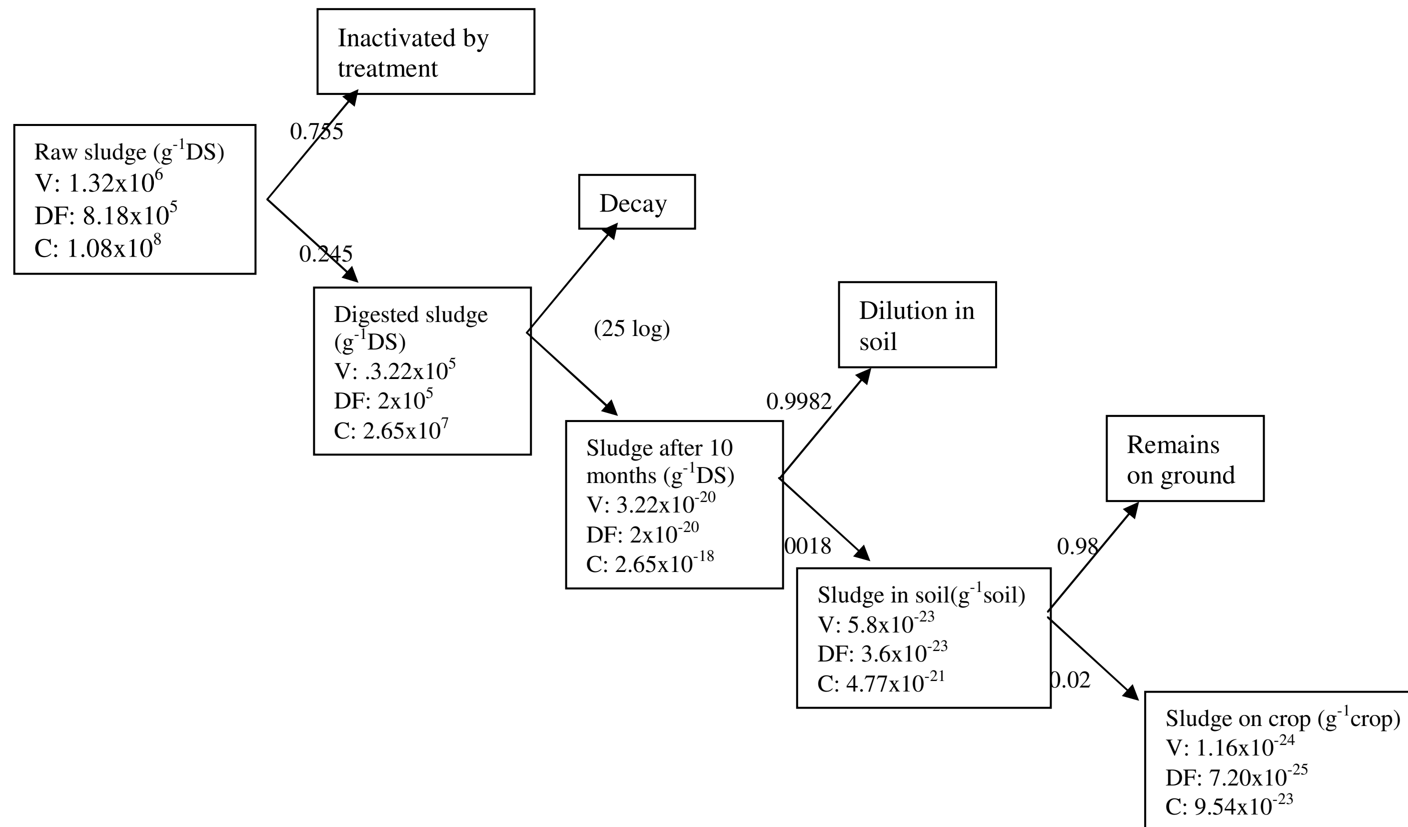
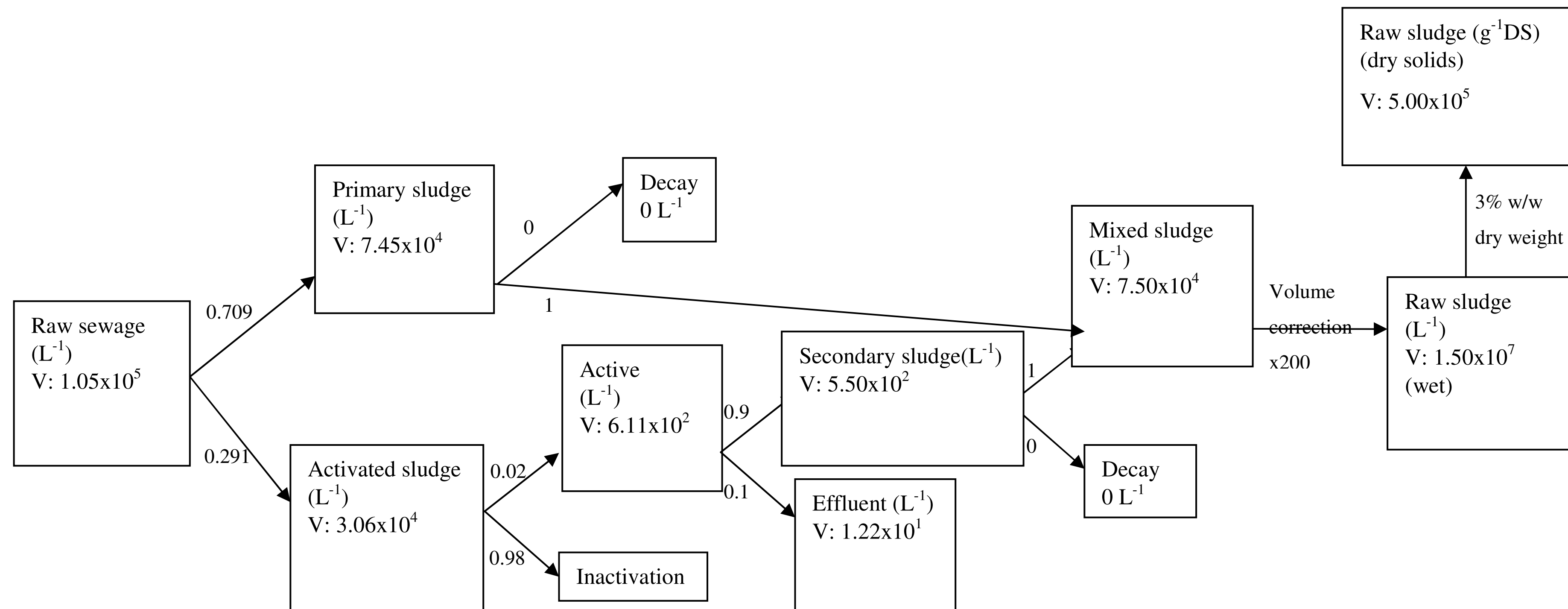


Figure C.2. Microbiological event tree for rotavirus transfer and decay from raw sewage sludge to sludge crop loading (V – Victoria, DF – Distrito Federal, Mexico; C – Chiapas, Mexico)

Appendix D. *Campylobacter* risk assessment



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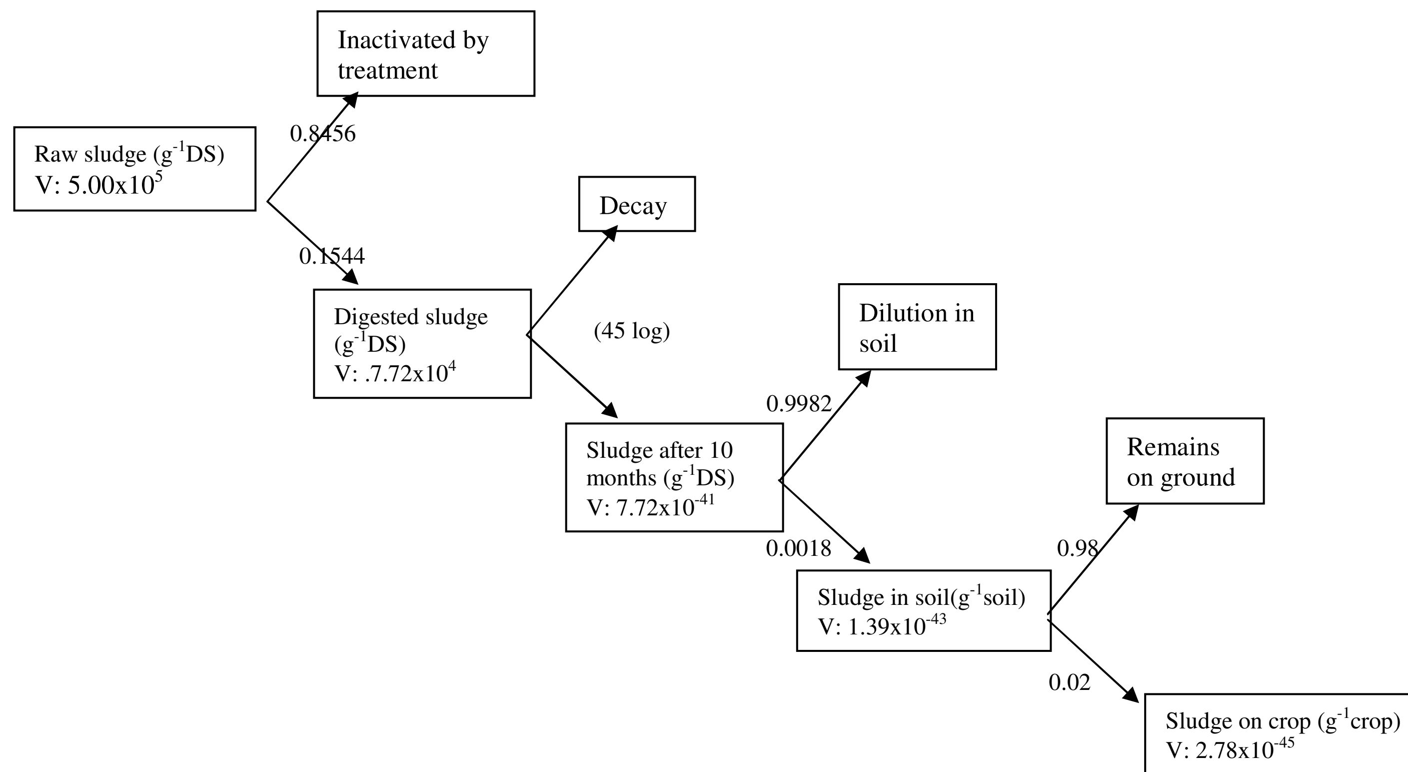

Figure D.2. Microbiological event tree for *Campylobacter* transfer and decay from raw sewage sludge to sludge crop loading (V – Victoria, DF – Distrito Federal, Mexico; C – Chiapas, Mexico)

Appendix E. *Salmonella* risk assessment

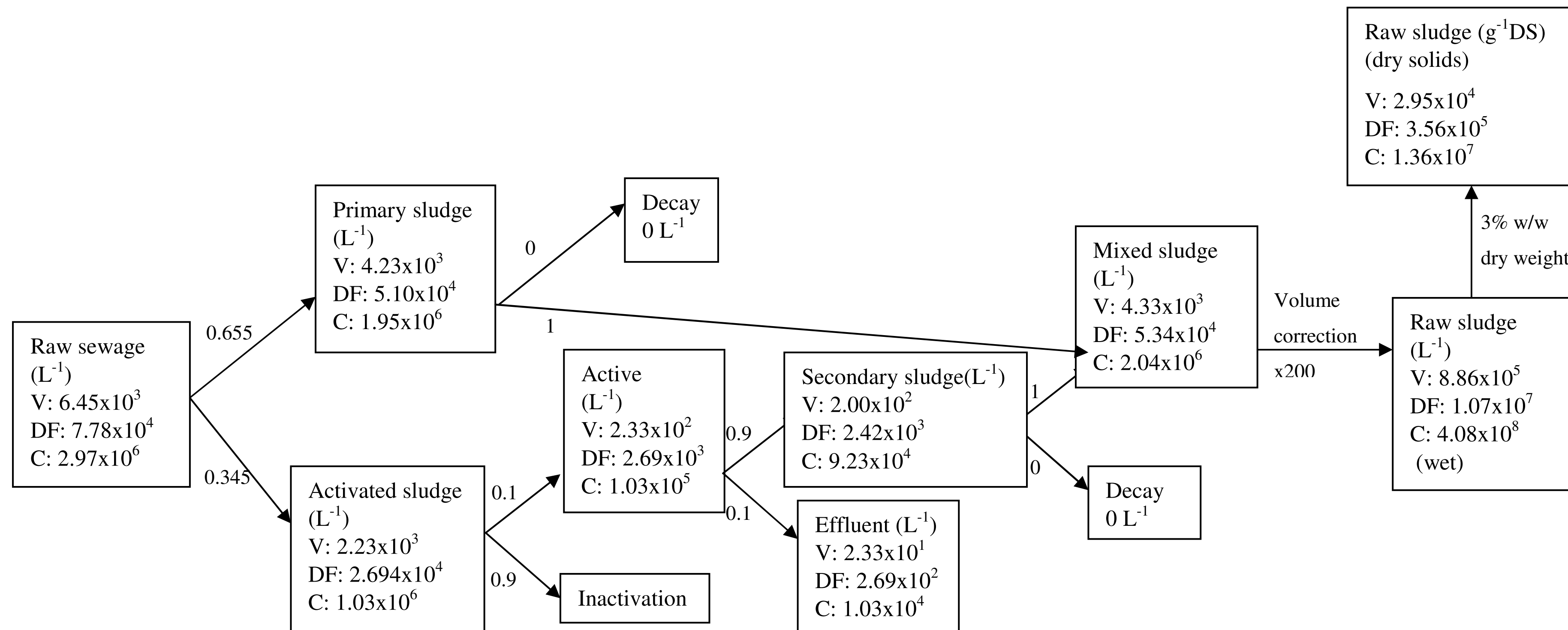

Figure E.1. Microbiological event tree for *Salmonella* transfer and decay from raw sewage to raw sewage sludge (V – Victoria, DF – Distrito Federal, Mexico; C – Chiapas, Mexico)

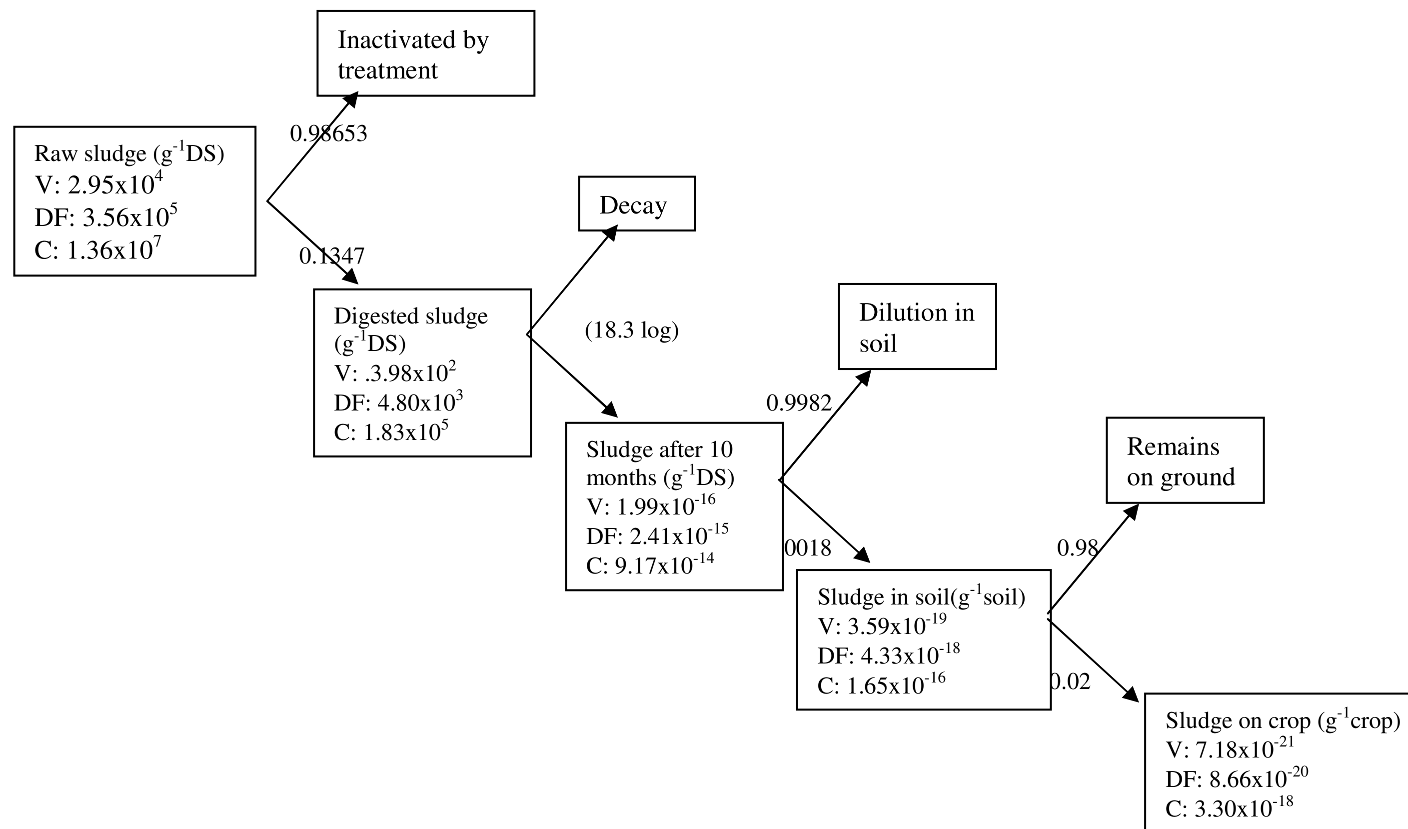

Figure E.2. Microbiological event tree for *Salmonella* transfer and decay from raw sewage sludge to sludge crop loading (V – Victoria, DF – Distrito Federal, Mexico; C – Chiapas, Mexico)

Appendix F. *Shigella* risk assessment

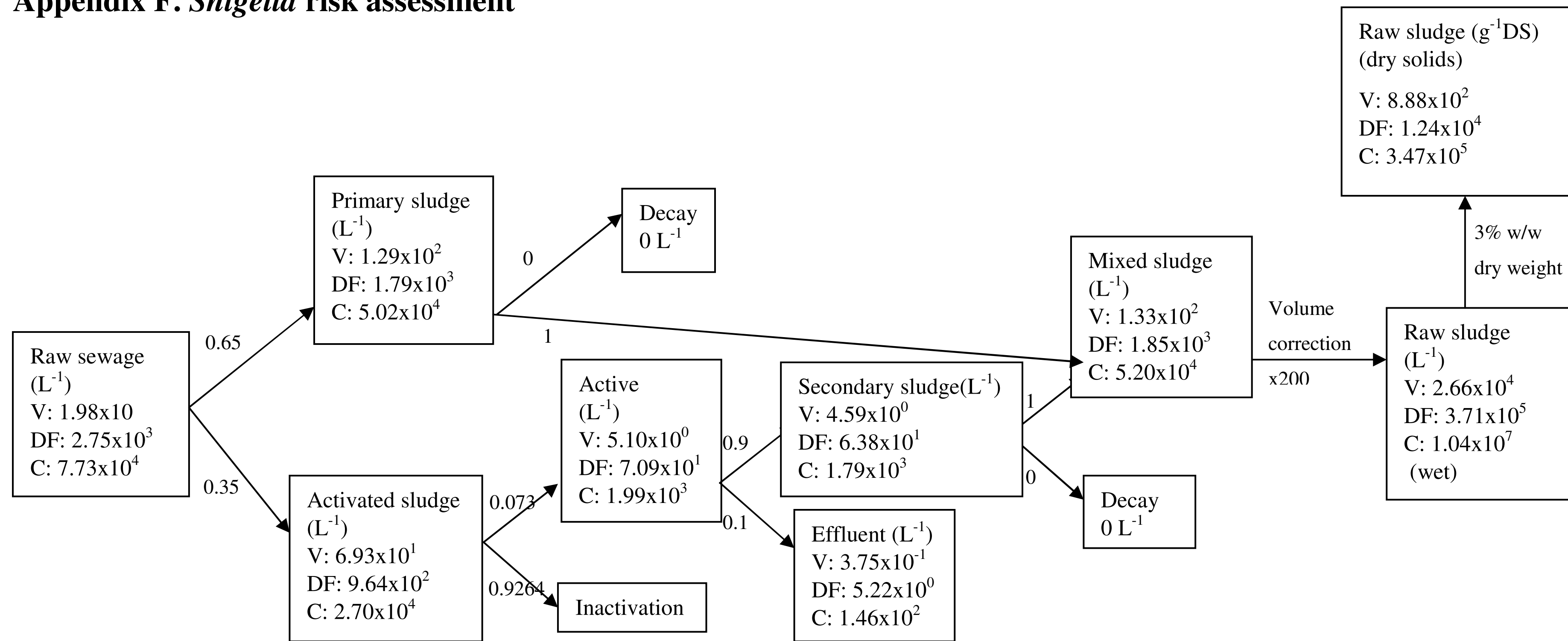

Figure F.1. Microbiological event tree for *Shigella* transfer and decay from raw sewage to raw sewage sludge (V – Victoria, DF – Distrito Federal, Mexico; C – Chiapas, Mexico)

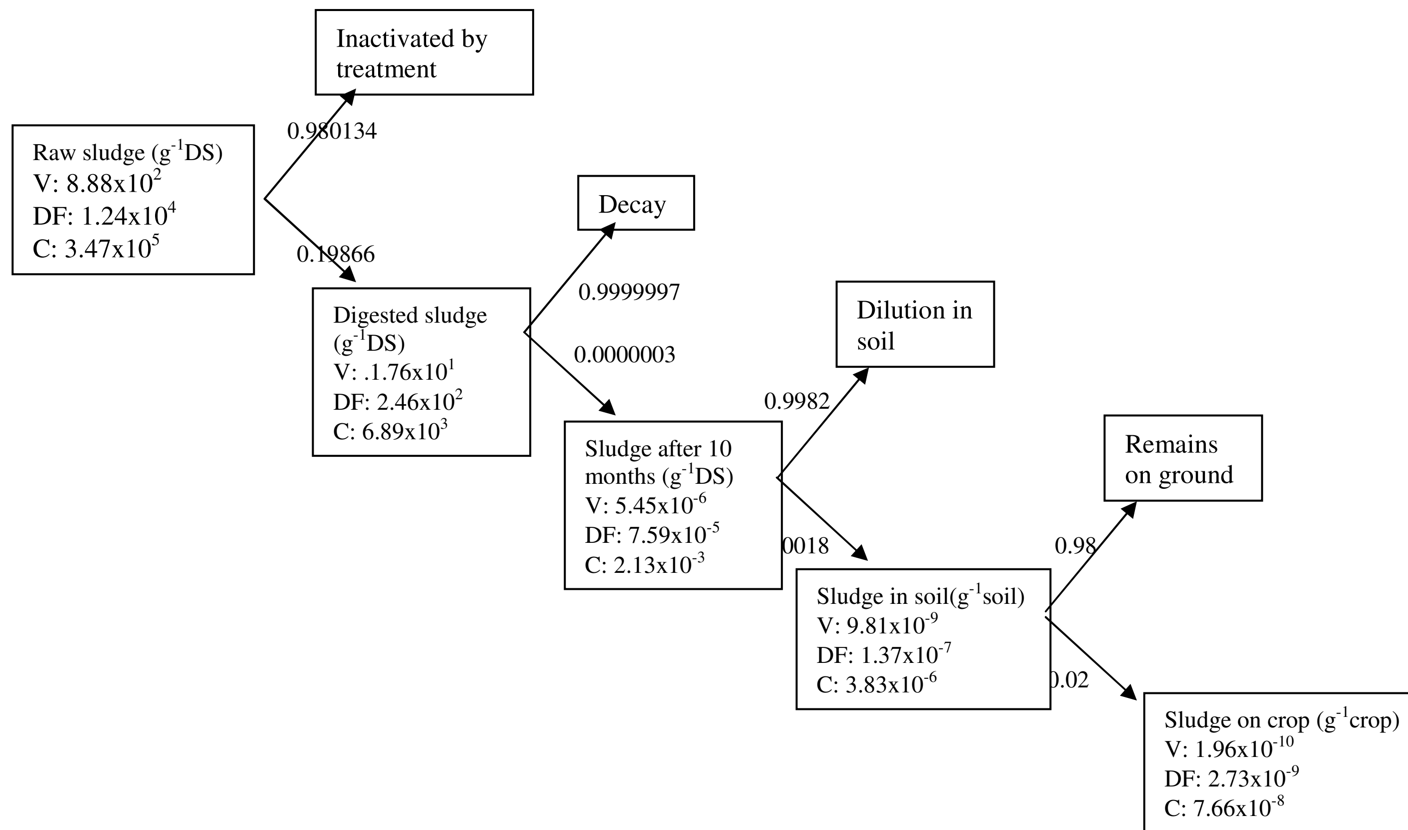

Figure F.2. Microbiological event tree for *Shigella* transfer and decay from raw sewage sludge to sludge crop loading (V – Victoria, DF – Distrito Federal, Mexico; – Chiapas, Mexico)

Appendix G. *Cryptosporidium* risk assessment

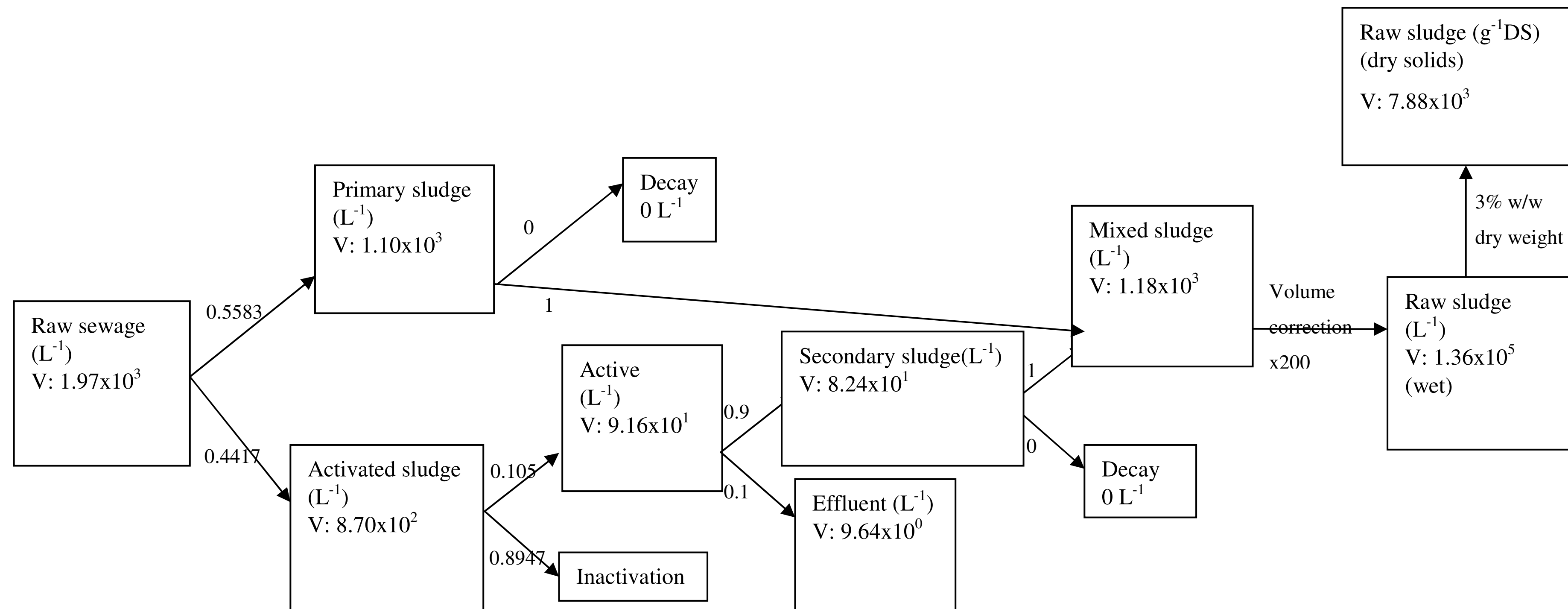

Figure G.1. Microbiological event tree for *Cryptosporidium* transfer and decay from raw sewage to raw sewage sludge (V – Victoria, DF – Distrito Federal, Mexico; C – Chiapas, Mexico)

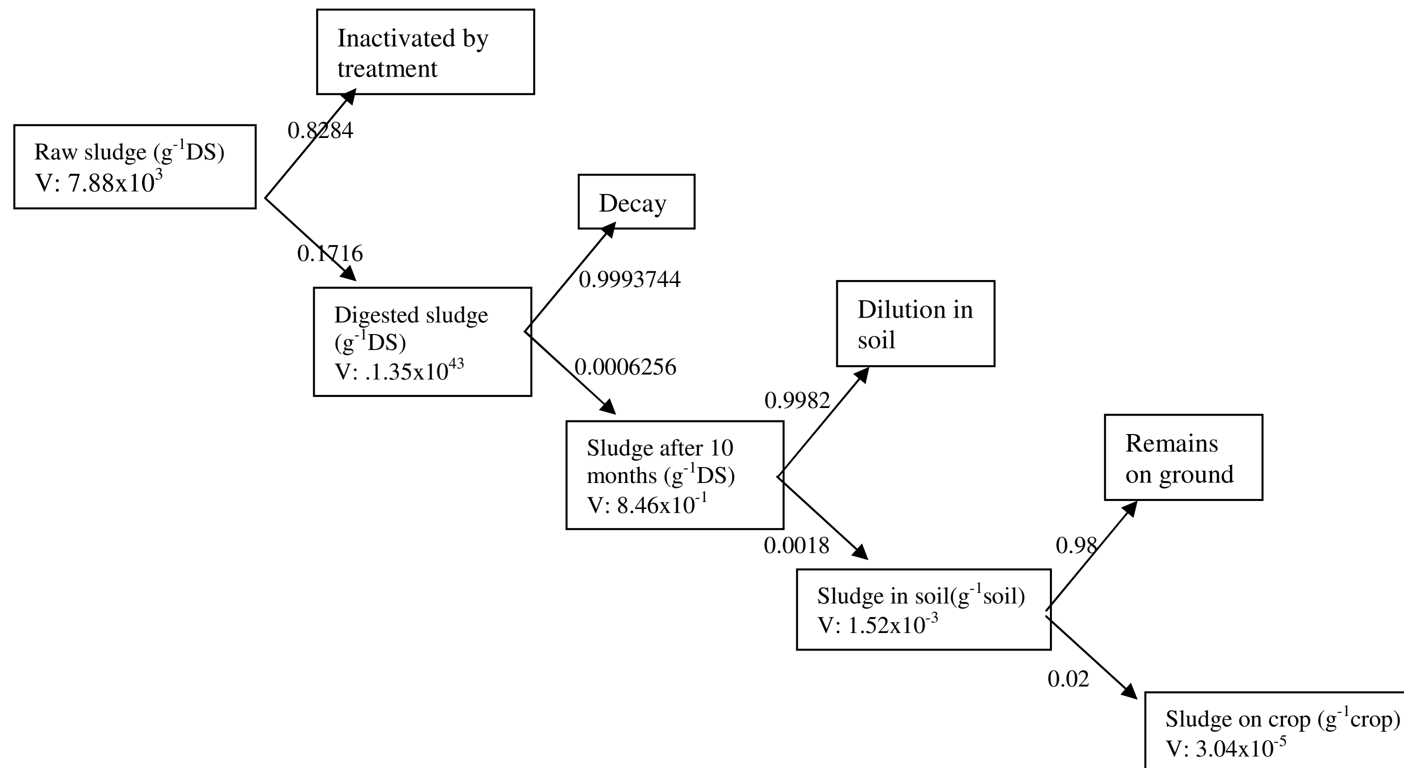

Figure G.2. Microbiological event tree for *Cryptosporidium* transfer and decay from raw sewage sludge to sludge crop loading (V – Victoria, DF – Distrito Federal, Mexico; – Chiapas, Mexico)

Appendix H. *Giardia* risk assessment

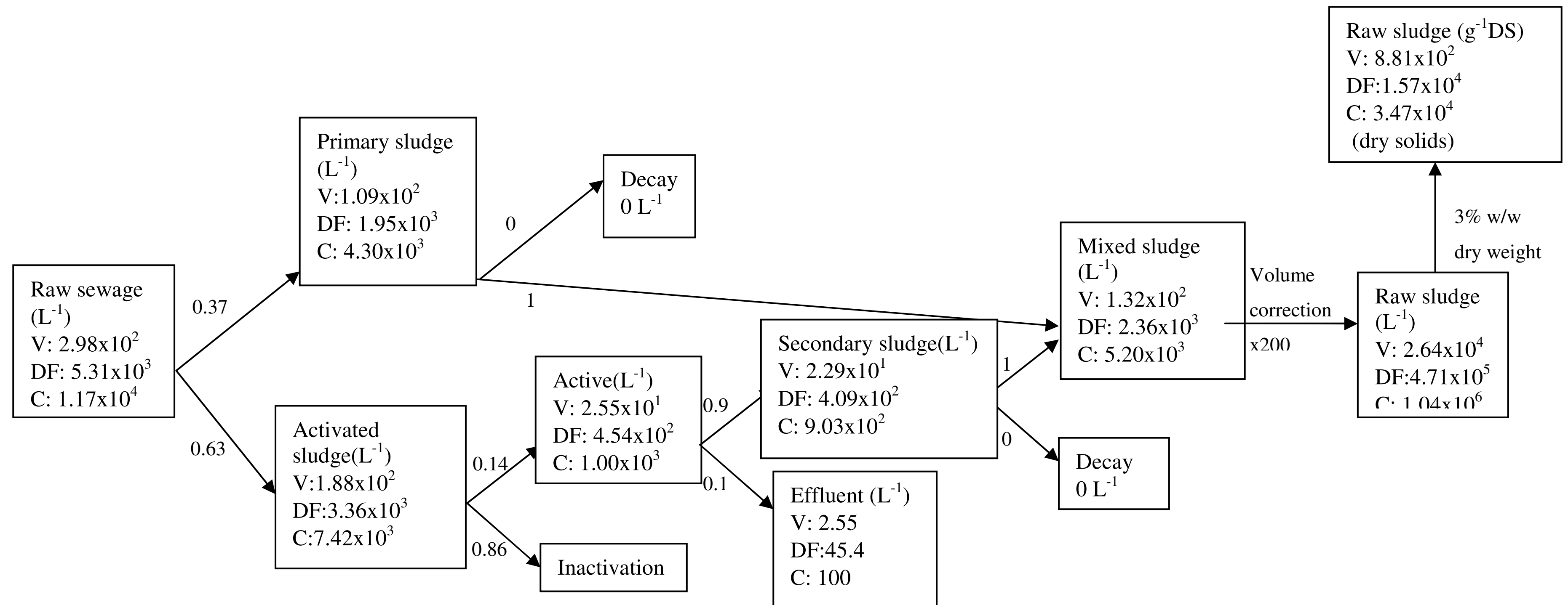

Figure H.1. Microbiological event tree for *Giardia* transfer and decay from raw sewage to raw sewage sludge (V – Victoria, DF – Distrito Federal, Mexico; C – Chiapas, Mexico)

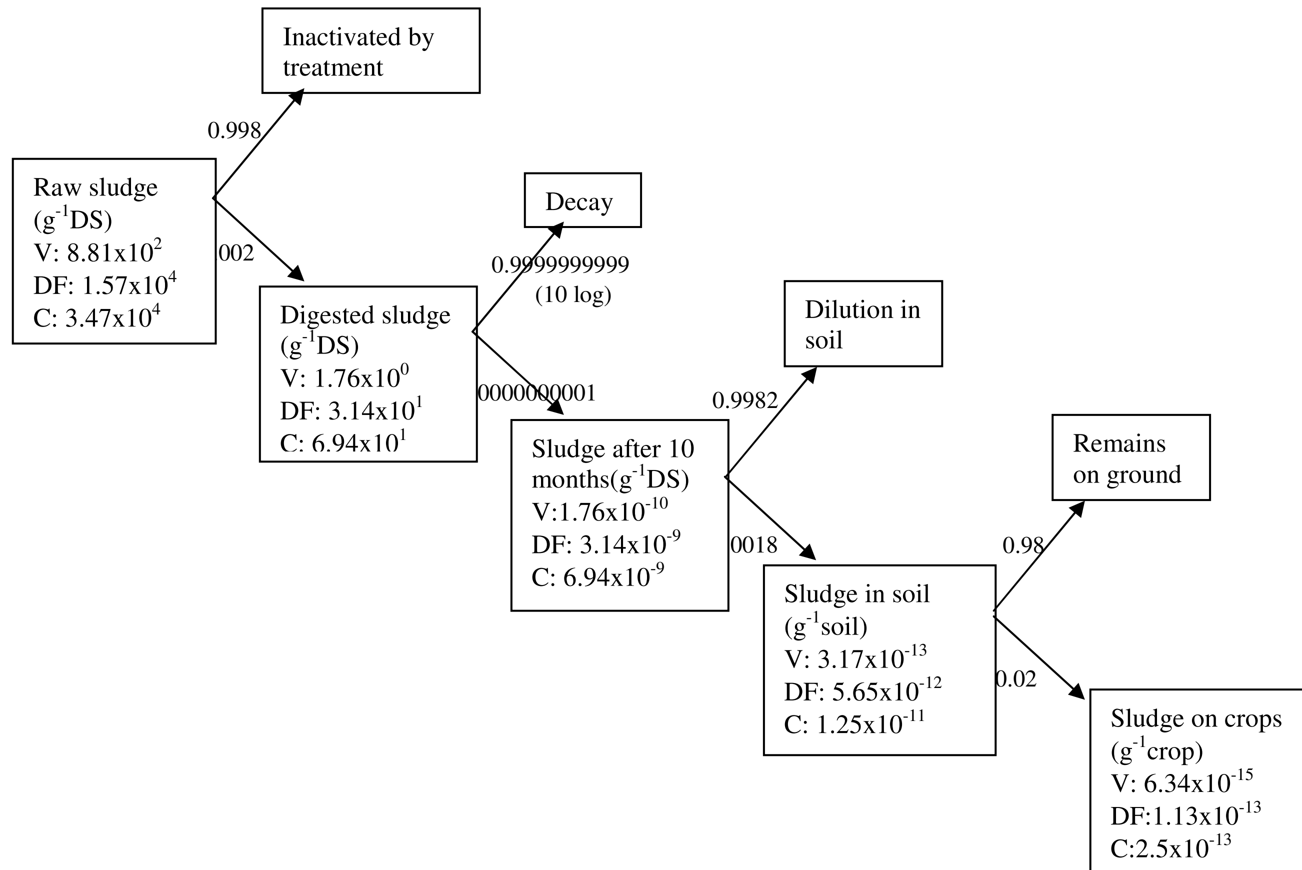

Figure H.2. Microbiological event tree for *Giardia* transfer and decay from raw sewage sludge to sludge crop loading (V – Victoria, DF – Distrito Federal, Mexico; – Chiapas, Mexico)

Appendix I. *Ascaris* risk assessment

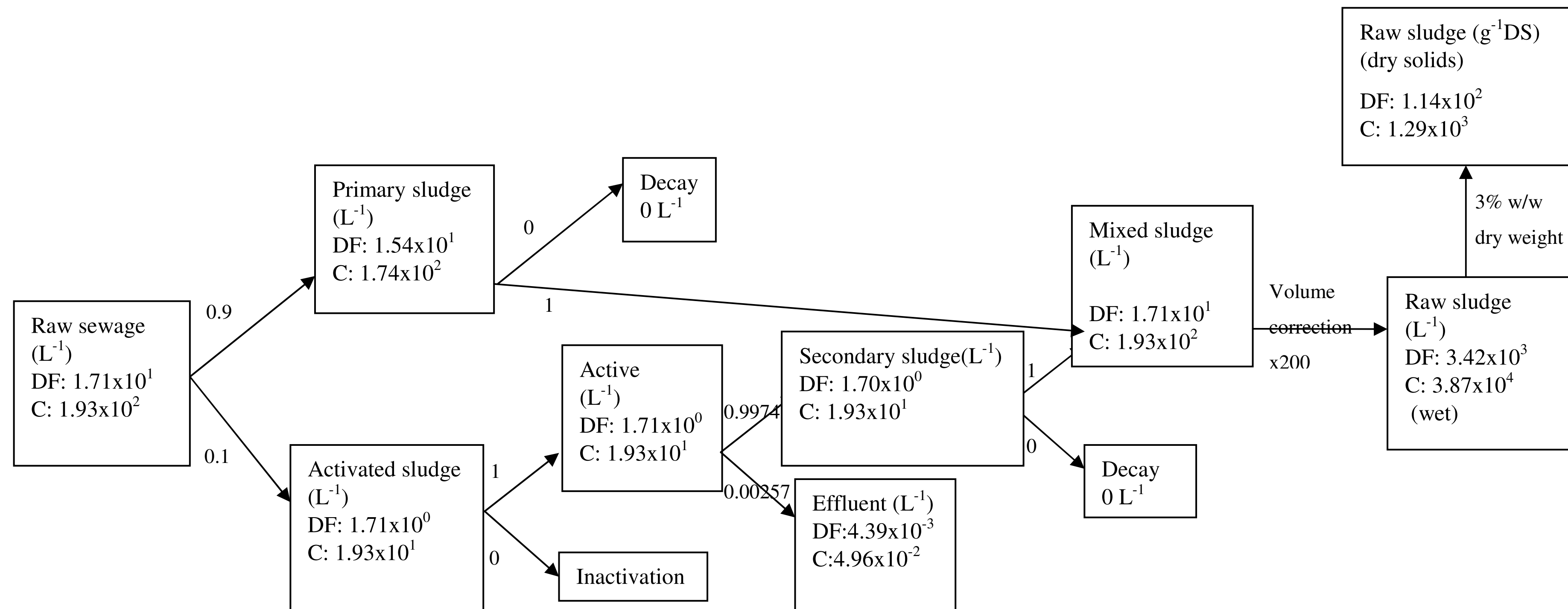

Figure I.1. Microbiological event tree for *Ascaris* transfer and decay from raw sewage to raw sewage sludge (V – Victoria, DF – Distrito Federal, Mexico; C – Chiapas, Mexico)

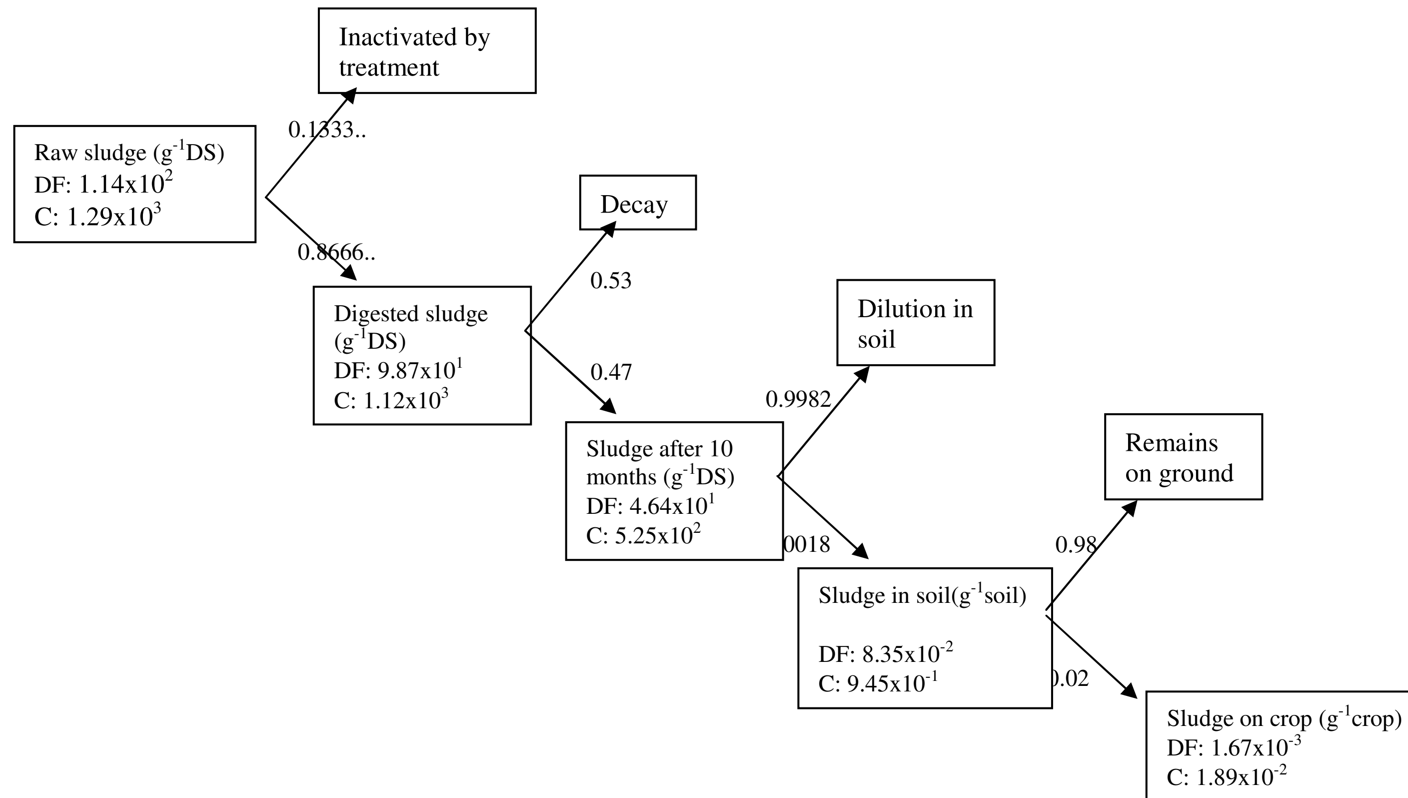

Figure I.2. Microbiological event tree for *Ascaris* transfer and decay from raw sewage sludge to sludge crop loading (V – Victoria, DF – Distrito Federal, Mexico; – Chiapas, Mexico)

Appendix J. *Taenia* risk assessment

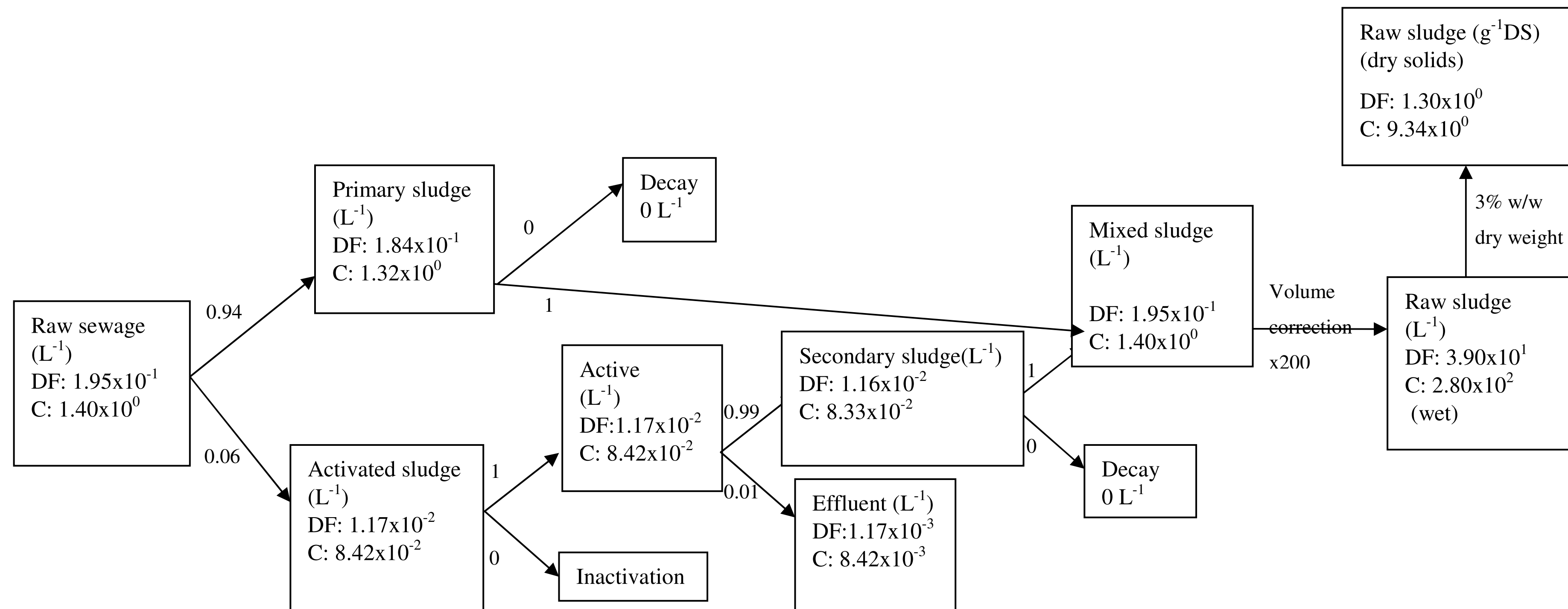

Figure J.1. Microbiological event tree for *Taenia* transfer and decay from raw sewage to raw sewage sludge (V – Victoria, DF – Distrito Federal, Mexico; C – Chiapas, Mexico)

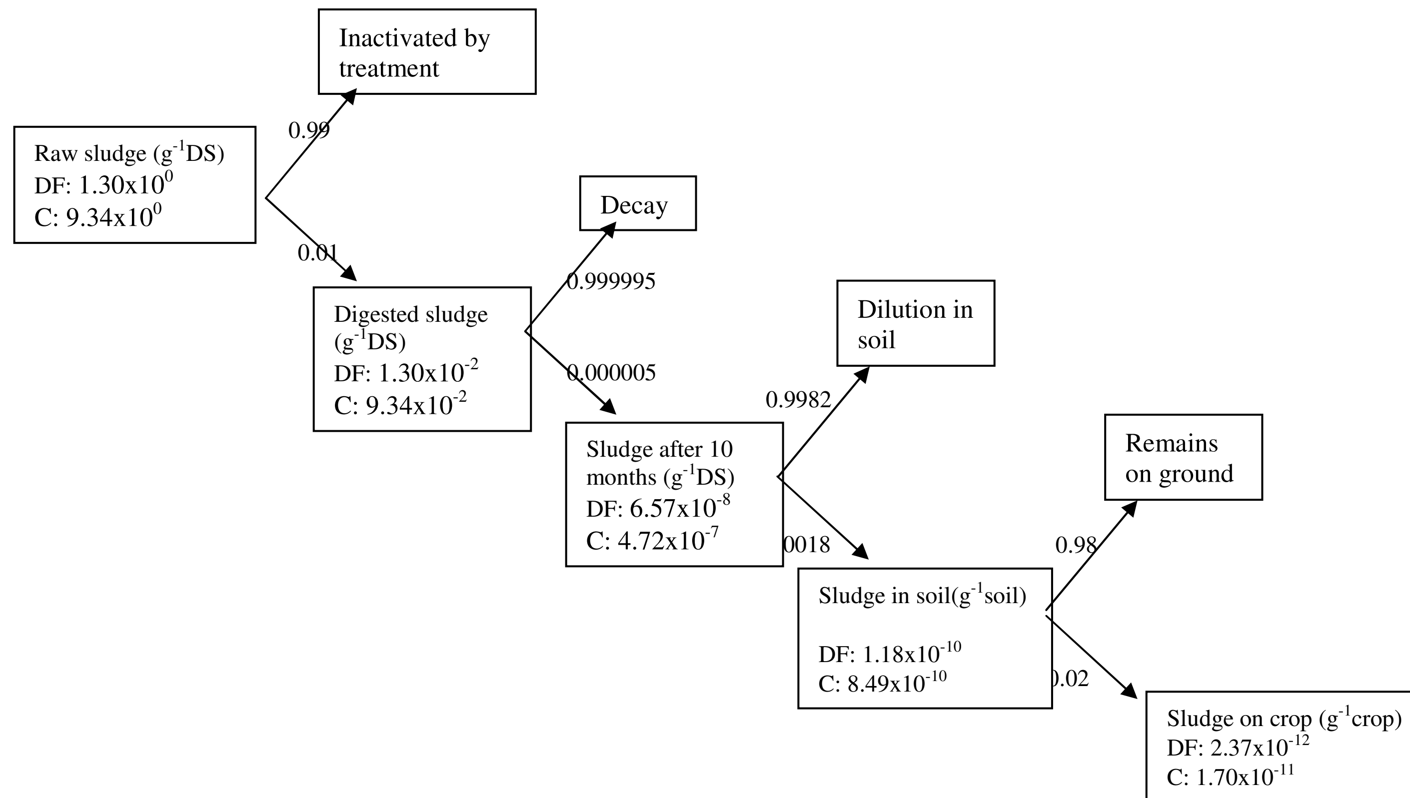

Figure J.2. Microbiological event tree for *Taenia* transfer and decay from raw sewage sludge to sludge crop loading (V – Victoria, DF – Distrito Federal, Mexico; – Chiapas, Mexico)

Appendix K. Sensitivity analysis for *Ascaris* – first iteration for one infection

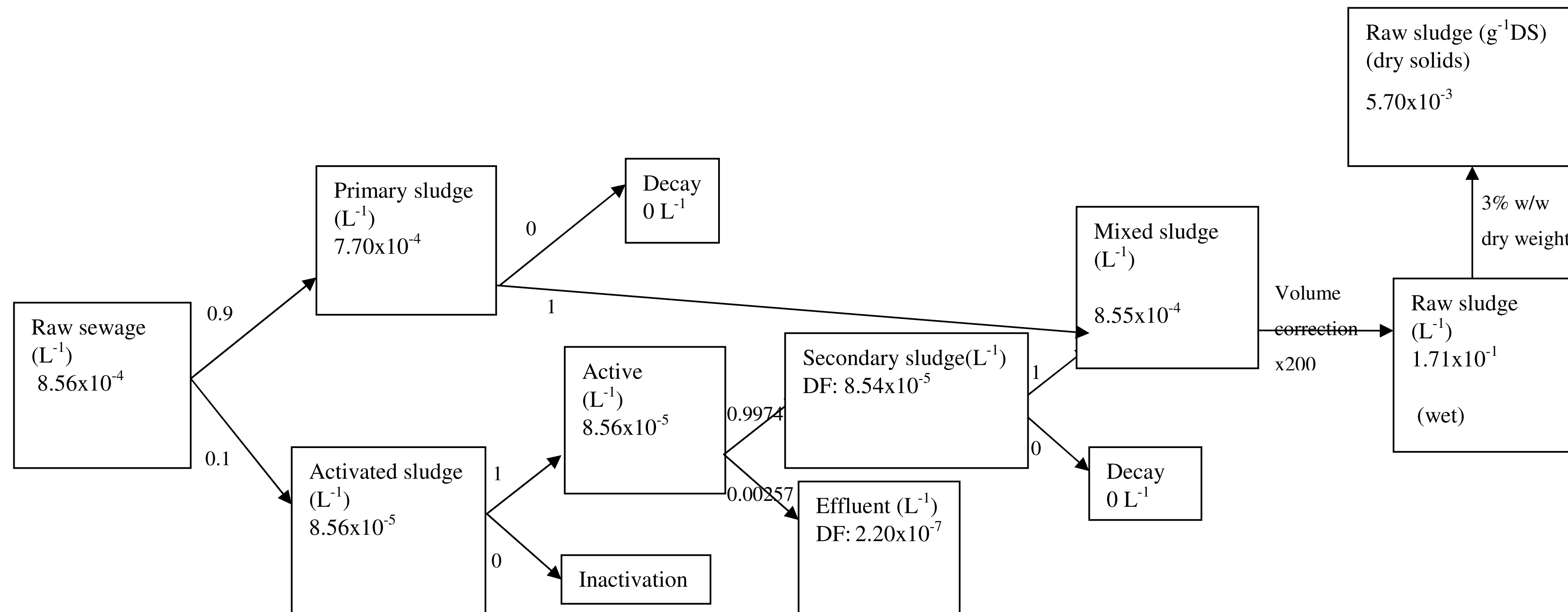

Figure K.1. Microbiological event tree for *Ascaris* transfer and decay from raw sewage to raw sewage sludge (V – Victoria, DF – Distrito Federal, Mexico; C – Chiapas, Mexico)

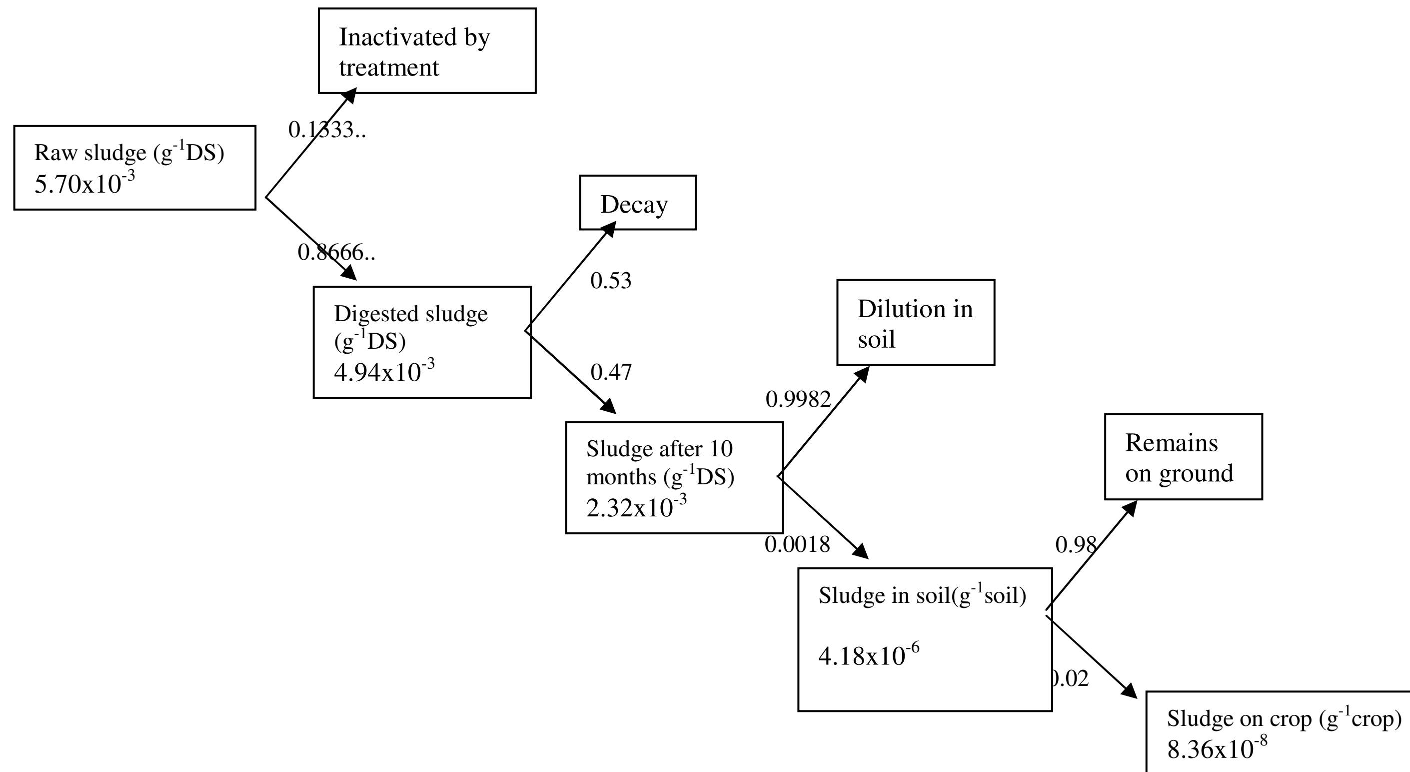

Figure K.2. Microbiological event tree for *Ascaris* transfer and decay from raw sewage sludge to sludge crop loading (V – Victoria, DF – Distrito Federal, Mexico; – Chiapas, Mexico)

Appendix L. Sensitivity analysis for *Taenia* – first iteration for one infection

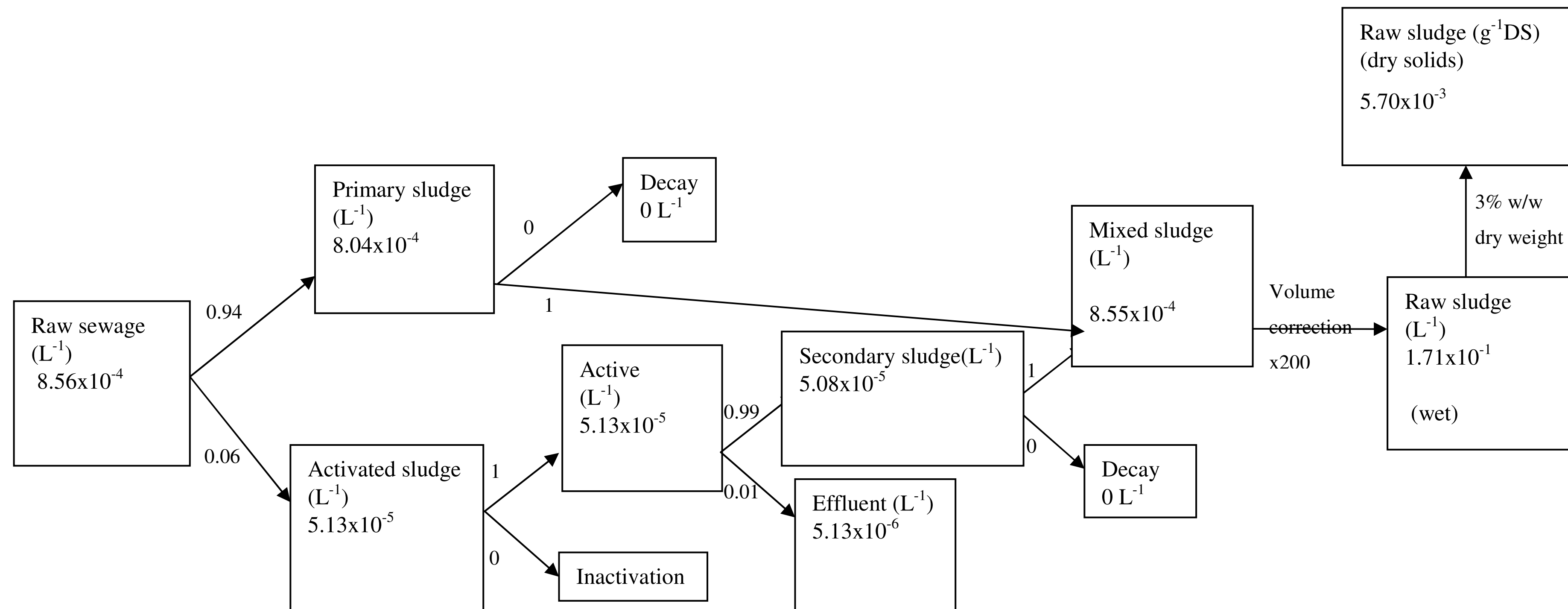

Figure L.1. Microbiological event tree for *Taenia* transfer and decay from raw sewage to raw sewage sludge (V – Victoria, DF – Distrito Federal, Mexico; C – Chiapas, Mexico)

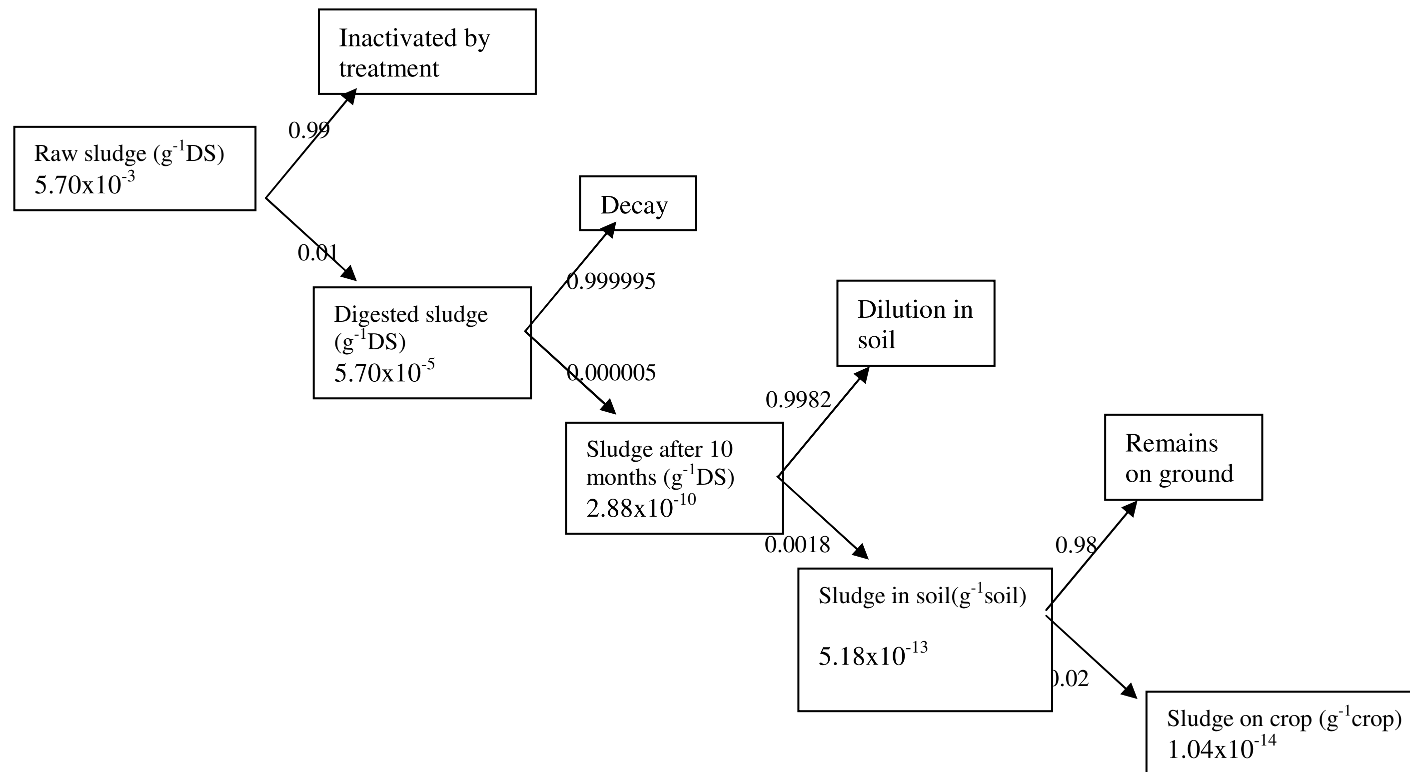

Figure L.2. Microbiological event tree for *Taenia* transfer and decay from raw sewage sludge to sludge crop loading (V – Victoria, DF – Distrito Federal, Mexico; – Chiapas, Mexico)