QMRA to inform the approach for regulating pathogens as part of the NSW Biosolids Guidelines Review

Final Report

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Contents

Glossary

ADWG: Australian Drinking Water Guidelines (NHMRC, 2011)

AGWR: Australian Guidelines for Water Recycling (NRMMC and EPHC, 2006)

Biosolids: an organic product derived from treated sewage sludge[1].

Colony forming units (CFU): a unit of measurement for microorganisms that can be grown in the laboratory and refers to the number of colonies successfully cultured from a certain sample size.

Critical Control Points (CCPs): locations in a process where a certain hazard can be controlled, either through total prevention, elimination, or reduction.

Critical pathogen concentration: the maximum allowable pathogen concentration in biosolids for a particular exposure pathway, given the safety target.

DALY: Disability Adjusted Life Year. Population metric of life years lost to disease due to both morbidity and mortality.

DAPI: 4',6-diamidino-2-phenylindole (DAPI) is a fluorescent stain that can pass through an intact cell membrane and reveal information about internal structures. Once a *Cryptosporidium* oocyst or *Giardia* cyst has been identified by microscopy, DAPI staining is used as an indicator of (oo)cyst viability.

HACCP: Hazard Analysis and Critical Control Points is a safety system that identifies, evaluates, and controls hazards from source to exposure.

Health-based performance targets: the required level of treatment in LRV to achieve safety, also referred to as treatment targets.

LRV: Log_{10} reduction value is a measure to quantify pathogen reduction during treatment (1 = 90% reduction; 2 = 99% reduction).

PFU: Plaque forming units are a unit of measurement for microorganisms that multiply in the laboratory by infecting host cells and refers to the number of plaques (areas of dead host cells) following culture of a sample of known size.

PPE: Personal Protective Equipment including any clothing or device that can be used to prevent exposure such as gloves and masks.

QMRA: Quantitative Microbial Risk Assessment. Microbial risk assessment when each component in the model is specifically quantified.

[1] Definition currently under review by NSW EPA

Raw sewage: wastewater that includes household sewage prior to any form of treatment.

Raw sludge: the separated solids stream of the sewage treatment process, prior to any specific sludge treatment (i.e., digestion, lagoon or dewatering).

Reference pathogen: pathogen selected to represent a broader group of pathogens. If the system is designed to protect public health from the reference pathogen, then it is assumed that public health will be protected from all pathogens in the broader group.

Risk: the likelihood that a hazardous event occurs, and the severity or consequence of the hazard.

Sewage: mixture of human excreta and water used to flush the excreta from the toilet and through the pipes. May also contain water used for domestic purposes.

Sewage sludge: during sewage treatment, solids are separated from the sewage effluent. The waste stream that captures the solids is referred to as sewage sludge.

Treated sludge: sewage sludge following treatment at the wastewater facility. Treatment typically involves anaerobic digestion or lagoon storage.

Treatment target: the required level of treatment in LRV to achieve safety.

1. Introduction

Beneficial use of biosolids is of great value in the circular economy, however it is critical to ensure that human health is protected. A proportion of pathogens present in wastewater are transferred to the sludge, and can persist through sludge and biosolids treatment and, unless adequately controlled, may still be a risk to public health.

The level of exposure to biosolids and hence potential health risk depends upon how biosolids are applied, and the ultimate end-use. Not all biosolids uses require the highest level of treatment. It is important to ensure that treatment is appropriate to the end-use targets, and that these targets are as flexible as practicable.

To date, NSW guidelines for biosolids have relied on process train, and end-product verification testing to ensure that biosolids are fit-for-purpose. Finished product testing within a traditional verification framework has long been known to be inadequate for protection of public health from microbial agents (Bartram et al., 2001; WHO, 2006). Limitations with sampling and the inability to analyse for all specific agents of concern mean that consistent and timely safety cannot be ensured. As a result of this limitation, within the food and drinking water production systems, Hazard Analysis and Critical Control Points (HACCP) has been applied to ensure that the final product is safe (FAO, 1997; Havelaar, 1994; WHO, 2017a). Indeed, in Australia, management of food safety, drinking water safety and recycled water safety ((NRMMC and EPHC, 2006) and Appendix A) is undertaken within a risk management framework that includes HACCP (See Box 1). Good management and control of the critical process conditions are the foundation of ensuring that microbial safety targets are achieved. Finished product testing within this approach is a very specific and targeted approach to verification that compliments the overall safety framework. A risk management framework has been recommended for controlling microbial risks associated with various fractions from wastewater, including sludge and biosolids (Westrell et al., 2003; WHO, 2006), however this has not as yet been realised for biosolids management in the Australian context.

In this report, health-based performance targets for microbial agents were derived within a Quantitative Microbial Risk Assessment (QMRA) approach to support safe and beneficial reuse of biosolids. While treatment plants in our cities generate large amounts of biosolids for beneficial reuse, specific consideration needs to be given to the context and practises undertaken across all New South Wales. In regional NSW around 1.9 million people live in around 500 urban communities which rely on regional sewerage services. Of those systems half of them serve less than 10,000 people with 10% serving less than 1,500 people. Biosolids within these systems are treated in a range of ways intended for beneficial reuse, however the full potential benefit of biosolids reuse is not currently being realised. There is a need for a flexible approach to ensuring microbial safety that will allow for beneficial end-use applications for biosolids, with realistic and energy efficient treatment strategies that still protect public health.

This study was intentionally a screening level desktop study, drawing on existing data sources. Therefore, no specific data collection programs have been undertaken to date to inform the investigation. In 2016, NSW EPA commissioned a literature review to support the

review of the pathogen criteria of the guidelines. The final report (Deere, 2017) from that review provides important context and recommendations for this QMRA. Additional data sources include published studies in the academic literature; relevant international guidance from related contexts; existing data collected by the NSW EPA as part of other programs; and the analysis of data collected by Sydney Water and provided to support this investigation (Appendix C).

Box 1. Risk Management Framework

The risk management framework applied in the AGWR and the ADWG consists of 12 elements organised into four main areas. In the context of recycled water, the framework is applied as illustrated in Figure 1

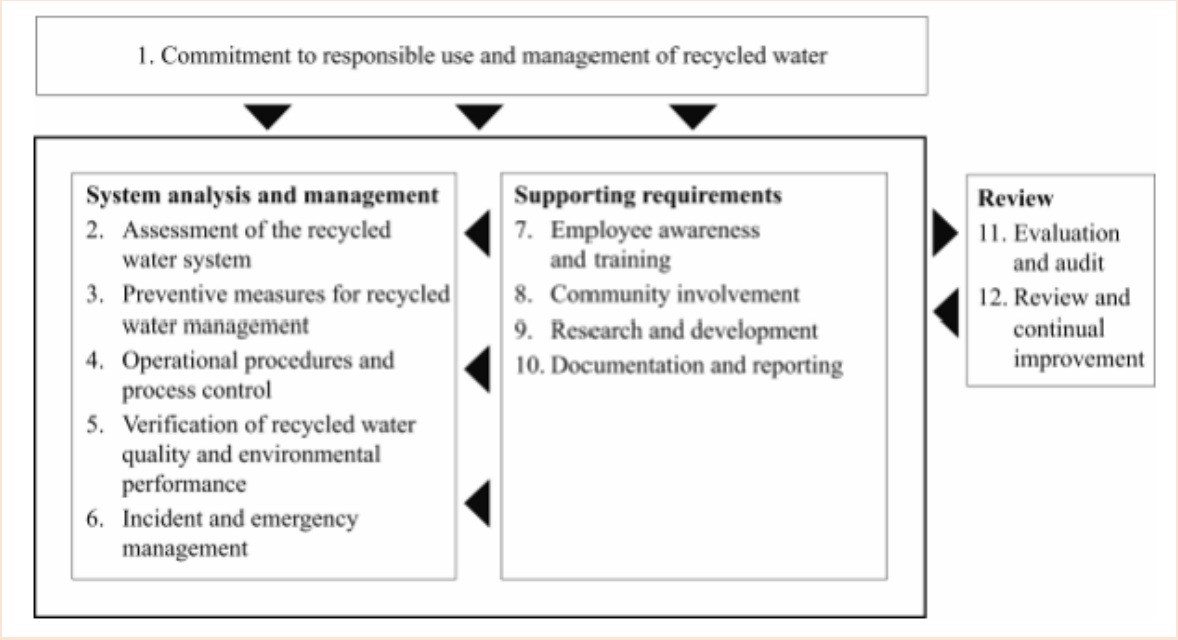


Figure 1. Elements of the framework for management of recycled water quality and u

Commitment. This requires the development of a commitment to responsible use of recycled water and to application of a preventative risk management approach to support use. The commitment requires active participation of senior managers, and a supportive organisational philosophy within agencies responsible for operating and managing recycled water schemes.

System analysis and management. This requires an understanding of the entire recycled water system, the hazards and events that can compromise recycled water quality, and the preventive measures and operational controls necessary for assuring safe and reliable use of recycled water

Supporting requirements. These include basic elements of good practice, such as employee training, community involvement, research and development, validation of process efficacy, and systems for documentation and reporting.

Review. This includes evaluation and audit processes to ensure that the management system is functioning satisfactorily. It also provides a basis for review and continuous improvement.

2. Approach

2.1. Defining quantitative treatment targets

The QMRA framework consists of four steps (WHO, 2016):

Problem formulation: the purpose and scope of the problem is defined. This scope includes the hazards to be considered, the exposure pathways and health endpoints.

Exposure assessment: each component of the exposure pathway is quantified based on the best available scientific evidence.

Health effects assessment: the probability of infection, illness and subsequent health impacts are evaluated based on the best available scientific evidence.

Risk characterisation: the targets for the risk assessment are then quantified relying on the previous three steps.

Application of this approach for defining treatment targets for biosolids is illustrated in Figure 2. Beginning with the concentration of pathogens in raw sewage, the fate, transport and persistence through the sewage and sludge treatment processes are quantified. This approach allows for the concentration of pathogens in the finished product to be linked to the operational conditions (e.g. residence time, temperature, pH) of the treatment processes. Depending on the end-use and associated estimated exposure to biosolids, the probability of infection and illness can be quantified and compared with the health outcome target. Treatment and exposure controls required to achieve safety can then be quantified in terms of Log_{10} reduction value (**LRV**) in pathogen concentration. Within this framework, based on any defined end-use application, the required performance targets can be defined to ensure safety.

2.2. Problem formulation

The scope of the investigation, in terms of the range of uses and exposure pathways to be considered, was defined in consultation with a stakeholder group through a problem formulation workshop. Key outcomes of the workshop are summarised in the following sections.

2.2.1. Selection of reference pathogens

Recommendations from (Deere, 2017) combined with the input from the problem formulation workshop led to the selection of the following reference pathogens:

Bacteria: *Salmonella* and *Campylobacter* were selected to represent bacterial pathogens. *Salmonella* is commonly selected as a reference pathogen for biosolids assessment due to the well documented potential for *Salmonella* regrowth in stored biosolids. Given that the Australian Guidelines for Water Recycling (AGWR) use *Campylobacter* as the reference pathogen, *Campylobacter* is also included in the assessment for comparison.

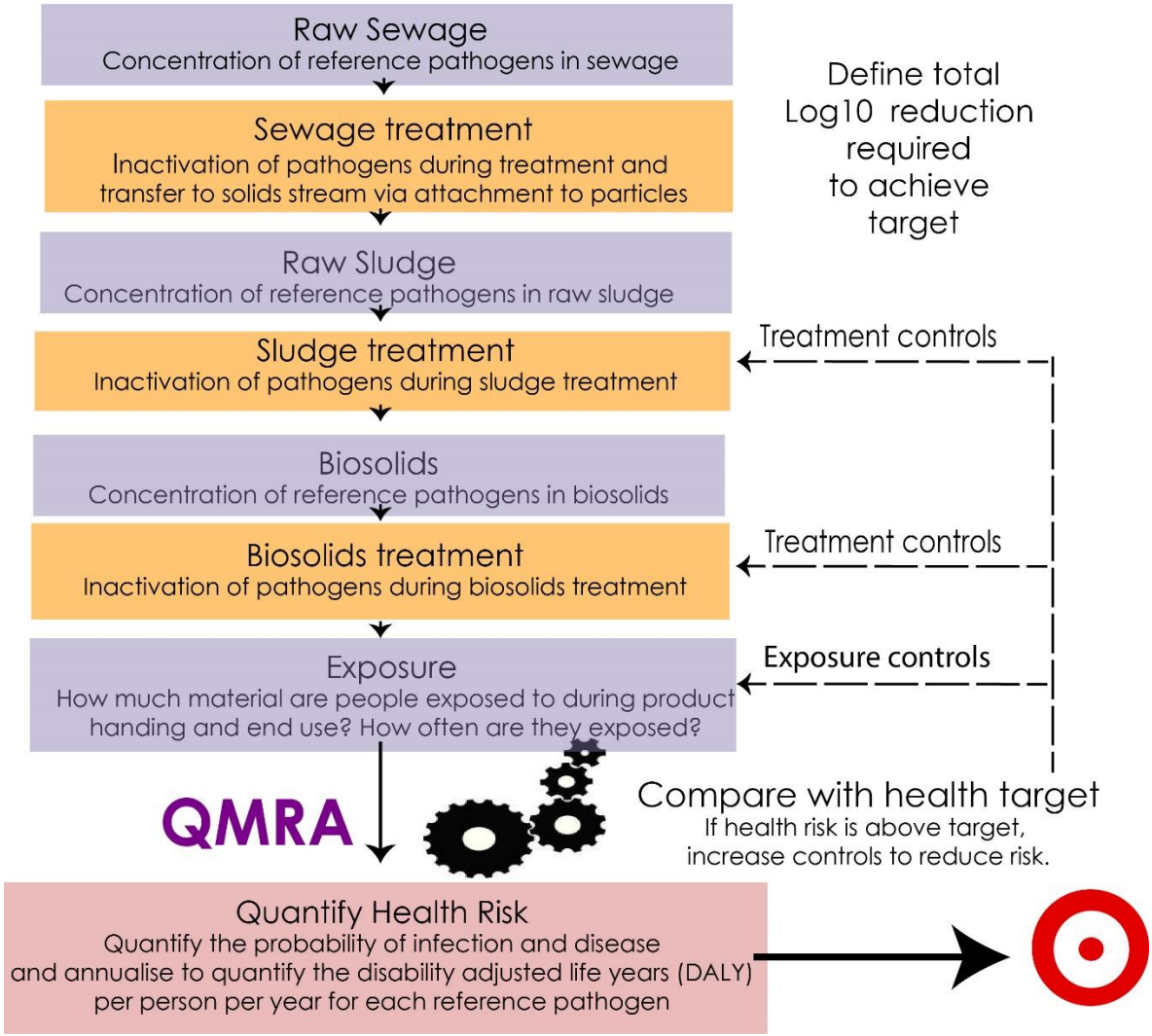


Figure 2. QMRA approach for defining health-based performance targets for beneficial reuse of biosolids

Virus: Adenovirus was selected to represent enteric viruses. Given the importance of identifying infectious viruses (as opposed to potentially inactivated viruses from molecular data) for the risk assessment, it was agreed to use a virus that can be cultured from biosolids. Adenovirus is present in high numbers in sewage and can be cultured from biosolids. Adenovirus can be supplemented with data from other viruses (e.g. dose-response and health impact data) for different components of the model. The result is a hybrid reference virus. A hypothetical hybrid virus has been applied extensively for defining guideline targets (including AGWR) to ensure that the best data is applied at each stage of the model, and the overall result is conservative for all human enteric viruses.

Protozoa: *Cryptosporidium* was selected to represent parasitic protozoa. *Cryptosporidium* is typically present in high numbers in sewage and has been frequently identified in sludge. *Cryptosporidium* oocysts can be highly infectious and lead to important health impacts. Relatively good epidemiological data is available to quantify health impacts. Selection of *Cryptosporidium* is consistent with AGWR and ADWG. Given that *Giardia* can be present in higher numbers in sewage (even though

less persistent than *Cryptosporidium*) it was decided to also include *Giardia* in the calculations for comparison.

Helminths: *Ascaris* (to represent helminthic pathogens that are directly transmissible); and *Taenia* (to represent helminthic pathogens that are indirectly transmissible) were selected to represent the soil transmitted helminths of concern via biosolids. It is also noted that based on the recommendations of Deere et al. (2017), these pathogens should be included on a case-by-case basis upon consideration of the catchment area and intended uses.

2.2.2. Selection of exposure pathways

The selection of exposure pathways was driven by the scope of the QMRA and focussed on human exposure and subsequent health risks. The end-uses were selected to illustrate the range of applications and is not intended to be exhaustive. The objective was to illustrate the implementation of the quantitative risk-based approach for setting safety targets, and how this approach compares with current guidance.

The selected pathways are illustrated in Figure 3 and include current allowable biosolids end-uses (see Table 3.6 of NSW guidelines (EPA, 1997)) for products achieving Stabilisation Grade B (sludge digestion/drying/stabilisation), and end-uses that currently require Grade A Stabilisation (thermal or pH treatment with digestion) or activity constraints with Stabilisation Grade B.

Pathways include:

- Agriculture including pasture improvement for grazing. (Stabilisation Grade B)
- Mine site rehabilitation. (Stabilisation Grade B)
- Public greenspace application. The use of biosolid products for soil condition and fertiliser in public parks and gardens, including golf courses. (Stabilisation Grade A)
- Residential use. The use of biosolids products by households for residential garden improvement. (Stabilisation Grade A)
- Pasture improvement for food crops, including above and below ground crops. (Stabilisation Grade B with Activity Constraints)
- Composting with green waste by contractors. (Stabilisation Grade A)

For a human health risk to exist, people must be exposed to the pathogen. Points in each exposure pathway were identified where people may be exposed to the biosolid product. These exposure groups include workers involved in transport and biosolids application, together with members of the public.

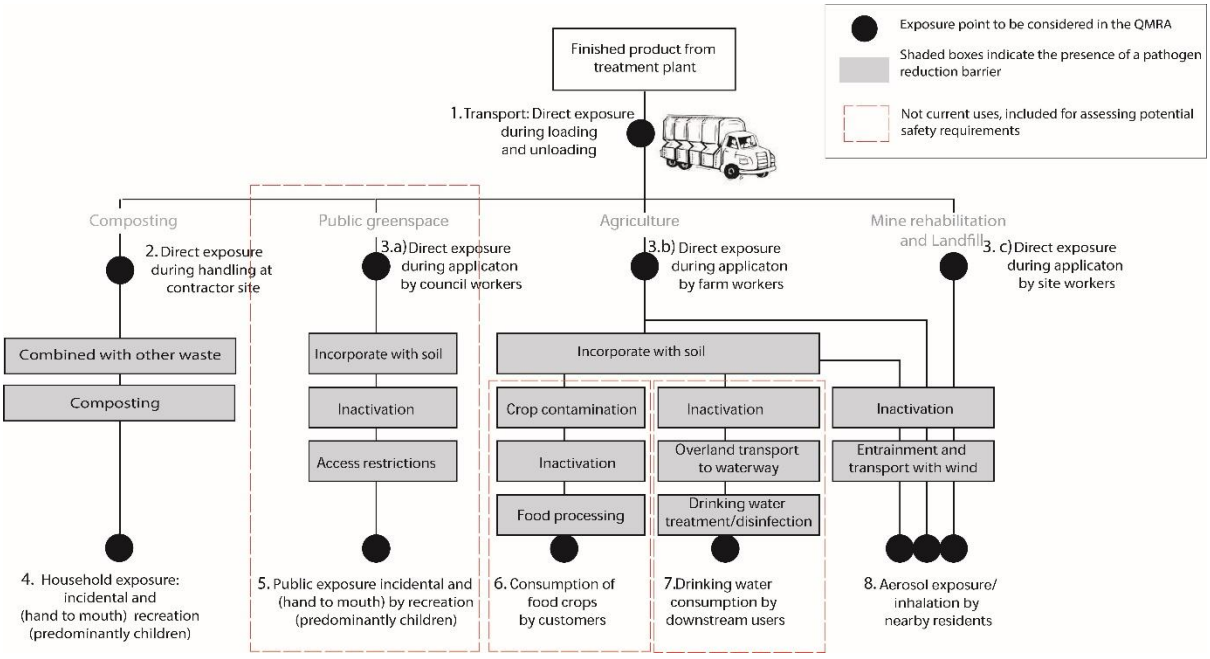


Figure 3. Exposure pathways included in the QMRA identifying exposure points and pathogen reduction barriers

2.2.3. Selection of health outcomes

The Disability Adjusted Life Year (DALY) was selected to be the risk assessment health outcome metric as it is currently used in the Australian recycled water (AGWR) and drinking water (ADWG) guidelines. The DALY is recommended by the WHO (Havelaar and Melse, 2003) and allows for different duration and severity of illness to be accounted for in determining safety (illustrated in Figure 4). In the context of public health where negligible risk is expected (e.g. drinking water) the DALY target for defining safety is 1×10^{-6} DALY per person per year (pppy).

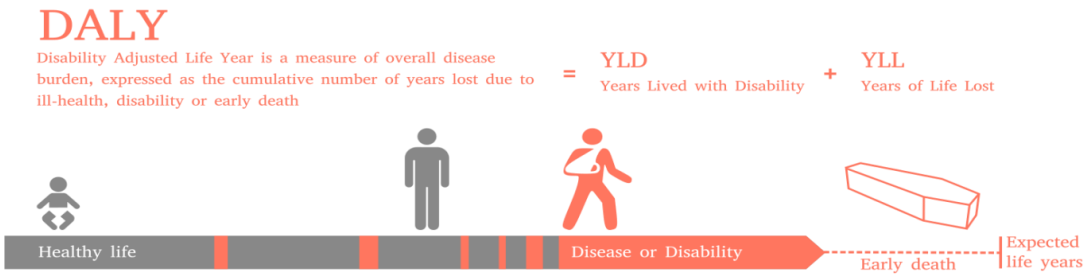


Figure 4. The Disability Adjusted Life Year (DALY) as a measure of disease burden

For intentional activities including residential application and workplace safety, the appropriate target is less clearly defined. Intentional activities provide the opportunity for additional controls to implemented to improve safety including personal protective equipment (PPE) (e.g. gloves and masks). All calculations will be undertaken with reference to the negligible risk safety target (1×10^{-6} DALY pppy), noting that in some contexts this could be partially achieved with PPE controls.

2.3. Exposure assessment

2.3.1. Define reference pathogen concentration in sewage, sludge and biosolids

Sewage

The first step in applying the framework (Figure 2) is to quantify the concentration of each reference pathogen in untreated sewage. The reported concentrations of human enteric pathogens in sewage is variable, spanning orders of magnitude (Rose and Jimenez-Cisneros, 2017; Soller et al., 2017). This variability depends on many factors including the incidence of disease in the population (only infected individuals excrete human pathogens); the dilution and dynamics of the wastewater system; the persistence of pathogens in wastewater; and the analytical methods used for enumeration in the laboratory (see Box 2).

BOX 2. Laboratory enumeration method and quantification of pathogen concentration in environmental media

Quantifying specific target pathogens from environmental samples is complex. Environmental samples are complex matrices comprising a diverse range of chemical and biological compounds that influence the performance of culture and molecular methods. The need to concentrate large samples (e.g. for relatively pure surface water samples, up to 100L) or dilute small samples (e.g. sewage and sludge samples) adds increased complexity. When interpreting a reported concentration, it is important to consider the multiple steps that may have been needed for sample preparation. Each step in the process of concentrating or purifying the sample prior to analysis is an opportunity for microorganisms or nucleic acid to be lost. Quantitative method controls are required to evaluate potential loss at each step of the process.

Microbial methods are targeted towards a specific characteristic of the microorganism and vary in their specificity for identifying viable (capable of causing infection), human-infectious organisms. Methods may be targeted toward:

a. Visual identification under the microscope based on characteristic morphological features (often using specific staining techniques).
b. Ability to reproduce (culture or plaque assay) under a set of specific conditions. Specific microorganisms can be selected from a large microbial population by using selective media and selective incubation conditions. For some pathogens (e.g. Adenoviruses) the time required for cell culture may be up to two weeks. Identification of pathogens by cell culture is often an *underestimate of the true human infectious population*, as not all human infectious pathogens will be successfully cultured in the laboratory environment.
c. Molecular methods (usually with amplification by PCR) are used to identify the presence of a particular sequence of genetic material in the sample. The presence of the target genetic material may indicate infectious pathogens, or evidence of previously infectious pathogens. Molecular methods therefore typically provide an *overestimate of the true human infectious population*.

Integrated methods have been developed to overcome the limitations of culture (b) and molecular (c) approaches. Integrated cell culture and PCR (ICC-PCR) involves first amplifying the concentration of pathogens in the sample through culture on a targeted host cell line, followed by identification of the target organism through PCR. The ICC-PCR has superior detection sensitivity for viruses in comparison to traditional cell culture methods, however the reported concentrations may still be an overestimate of the human infectious concentration (White, 2016).

For simplicity in the context of regulation, it is desirable to define a default point estimate for each reference pathogen. Table 1. includes three examples of default point estimates for reference pathogen concentration in raw sewage from different guidance documents: the Australian Guidelines for Water Recycling, the World Health Organization potable reuse guidelines and the California potable reuse guidelines. These default values provide a universal starting point for the implementation of the framework. They are intended to be conservative. Where site specific data is available that should still be favoured.

To define default reference pathogen concentrations in sewage that are relevant for NSW systems, local data was reviewed. Primary data from analysis of sewage and biosolids products at five Sydney Water water resource recovery facilities were analysed and reviewed. A summary of the study and analysis of the results is included as Appendix C. Additional published studies undertaken in NSW were considered.

Table 1. Default reference pathogen concentrations in raw sewage from guideline documents

	Campylobacter (.L-1)	Enteric viruses vu.L-1	*Cryptosporidium* (oocysts.L-1)	*Giardia* (cycst.L-1)
Australian Guidelines Water Recycling (default 95th Percentile) (NRMMC and EPHC, 2006)	7 000	8 000 (adenovirus)	2 000	
WHO (potable reuse of wastewater) (default 95th Percentile) (WHO, 2017b)	7 000	20 000 (norovirus)	2 700	
California, USA default concentration (maximum) (Olivieri et al., 2016)*		100 000	10 000	100 000

*These values are deliberately very conservative and based on worst case assumptions with limited data (David Cunliffe pers comm)

Sludge

Pathogen concentrations in raw sludge were estimated by numerical modelling using the approach proposed by (Gale, 2003) illustrated in Figure 5. The approach relies upon model parameters accounting for partitioning of pathogens to solids and inactivation in secondary treatment. The selected values for these parameters are summarised in Table 2.

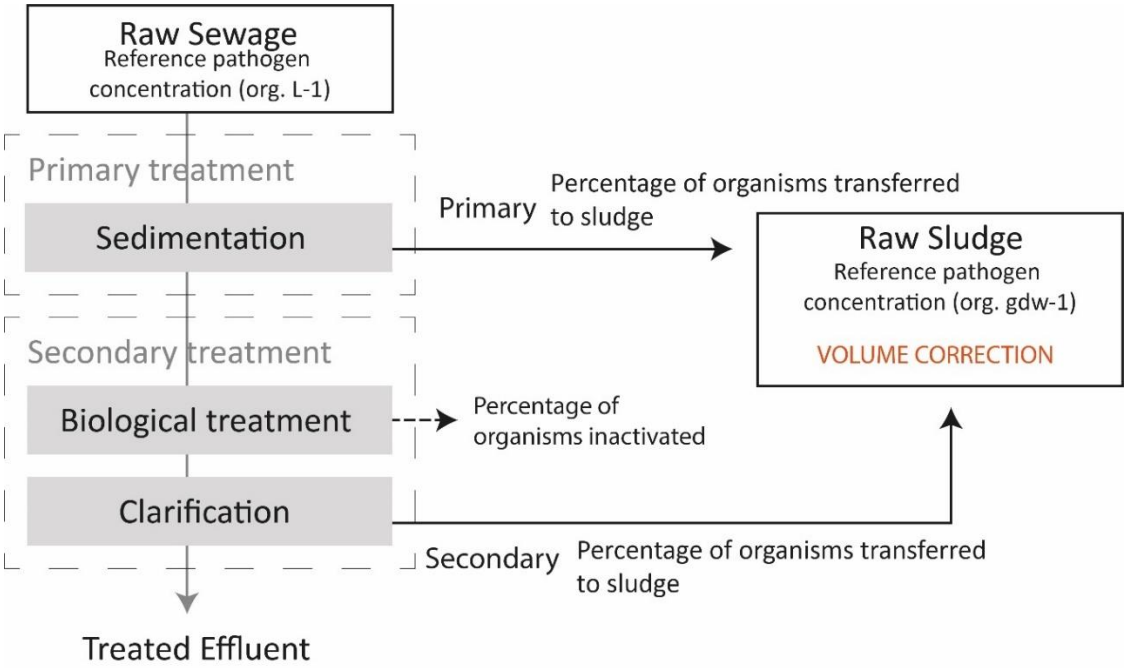


Figure 5. Event tree for partitioning of salmonellas into raw sewage sludge at a sewage treatment works (modified from (Gale, 2003)

Table 2. Fate and transport coefficients for pathogens in sewage treatment

	Campylobacter	*Salmonella*	Enteric viruses	*Cryptosporidium*	*Giardia*
Primary[1]					
Percentage of organisms transferred to solids	71	66	35	56	37
Secondary: activated sludge					
Percentage of organisms inactivated[2]	93	93	90	88	88
Secondary sedimentation[3]					
Percentage of organisms transferred to solids	90	90	90	90	90

Source: (Grant and Smith, 2010): [1] Table 5.7, Typical values [2]: Section 4.2.3; [3]: Table 4.5

2.3.2. Exposure magnitude and frequency by exposure pathway

Exposure pathways are illustrated in Figure 3. The magnitude of biosolids or biosolids amended soil that may be ingested or inhaled was estimated along with the frequency of each event. These values are summarised in Table 3.

2.4. Health impact assessment

The health impact assessment relies on a dose-response model, which is a mathematical relationship linking exposure with probability of infection or illness (see Box 3). Model parameters applied in the QMRA are summarised in Table 4 with comments on model selection in Table 5. Not all those who are infected will show symptoms of illness. Following quantification of infection probability, the likelihood of illness and subsequent disease burden is quantified. Parameters for the probability of illness given infection (P_{ill}), and DALY weightings used in the study are summarised in Table 6.

Table 3. Magnitude and frequency of exposure to biosolids by exposure pathway

Exposure pathway		Exposure group	Mass per exposure (g)	Frequency (per year)	Notes
1.	**Transport:** direct exposure during loading and unloading	Transport workers	0.05[1]	250	5 days per week
2.	**Composting facility**	Handling by workers	0.05[1]	250	5 days per week
3.	**Application:** application and incorporation	a) Council workers	0.05[1]	5	Seasonal
		b) Farm workers	0.05[1]	250	5 days per week
		c) Rehabilitation workers	0.05[1]	250	5 days per week
4.	**Household garden use**	Adults: incidental	0.033[2]	5	Seasonal
		Children: incidental	0.067[2]	5	Seasonal
		Children: hand to mouth	1	1	Single event of larger consumption
5.	**Public greenspace**	Adults: incidental	0.033[2]	5	Seasonal
		Children: incidental	0.067[2]	5	Seasonal
		Children: hand to mouth	1	1	Single event of larger consumption
6.	Consumption of **food crops**	Consumers	0.003[3]	100	
7.	**Aerosol** exposure/inhalation by nearby residents	Residents	0.05[1]	20	

[1]50mg per day to be consistent with the average outdoor soil and indoor dust incidental ingestion rates from (EnHealth, 2012) and incidental soil ingestion rates for low density residential in the NEPM (2013)
[2]Relying on assumptions applied for similar household use activities from Schonning et al. (2007)
[3]Residue on unwashed vegetables (NEPM, 2013)

Table 4. Dose-response models

	Dose-response Model	α	β	r*	r**	Reference
Campylobacter	Approx BP	0.145	7.59		0.019	(Medema et al., 1996)
	Exact BP	0.024	0.011		0.69	(Teunis et al., 2005)
Salmonella	Approx BP	8.53×10^{-3}	3.14		2.7×10^{-3}	(Teunis et al., 2010)
Norovirus	Exact BP	0.0044	0.002		0.69	(Messner et al., 2014)
Rotavirus	Exact BP	0.167	0.191		0.47	(Teunis and Havelaar, 2000)
Adenovirus	Exponential			0.42	0.42	(Crabtree et al., 1997)
	Exact BP	5.11	2.8		0.65	(Teunis et al., 2016)
Cryptosporidium parvum (combined)	Exponential			0.20	0.20	(Schijven et al., 2014a; WHO, 2017a)
Cryptosporidium hominis	Exact BP	8.37×10^{-11}	2.62×10^{-11}		0.76	(Schijven et al., 2014a)
Giardia	Exponential			0.02	0.02	(Teunis et al., 1996)
Ascaris	Approx BP	0.104	1.1		0.095	(Navarro et al., 2009)

r is the probability of infection associated with one microorganism r* published best-fit parameter of the exponential distribution, r** low-dose approximation parameter for the Approx BP and Exact BP models

BOX 3. Dose-response modelling

The dose-response model is a mathematical equation relating the dose of exposure to the probability of infection or illness. Models applied in QMRA typically take the form of a single-hit model, which means that each microorganism is assumed to act independently and has a certain probability (r) of passing a host's defences and achieving infection or illness. When r is a constant, the simplest form of the dose-response model is the result and is given by:

Exponential $P_{inf} = 1 - e^{-r.d}$

r is the probability that an individual microorganism will successfully achieve infection

d is the mean dose of microorganisms and is the product of the concentration in the exposure media and the amount of material (g or mL) consumed.

When *r* is allowed to vary according to a beta distribution (i.e., where r can take any value between 0 and 1), then the result:

Exact beta-Poisson $P_{inf} = 1 - {}_1F_1(\alpha, \alpha + \beta; -d)$

${}_1F_1$ is a confluent hypergeometric function.

Since the exact beta-Poisson model is extremely cumbersome, some studies have favoured an approximation of the beta-Poisson which holds true for certain parameter values of α and β, i.e. when $\alpha \ll \beta \; and \; \beta \gg 1$. When this condition is met, the complex model can be approximated to:

Approx beta-Poisson $P_{inf} = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha}$

The models summarised in Table 4 are published parameter estimates from the literature where authors have fitted the parameters of the single-hit model to human volunteer or outbreak data. Either the exponential or beta-Poisson model is typically chosen based on goodness of fit to the human data. While many models have been published, the ones used in this study are a selection of those considered to be most relevant for the current context of defining health-based treatment targets for biosolids.

Low-dose approximation

In the low-dose region (d < 0.1), the dose-response relationships are close to linear. When defining treatment targets to achieve safety, it is common to use as low-dose simplification to the single-hit model. In this case, the exponential model is used for all reference pathogens, where r is defined by:

$r = \frac{\alpha}{\alpha+\beta}$ for the exact BP; and

$r = \frac{\alpha}{\beta}$ for the BP approximation

While typically only one bacteria, virus and protozoa would be used to define health targets, in this case a broader range of pathogens and published input values were applied to test the sensitivity of the quantified LRVs to various health impact assumptions.

Table 5. Considerations in the selection of dose-response model to be used for quantifying treatment targets

Reference Pathogen	Comment on model selection for defining health-based targets
Campylobacter	Beta-Poisson approx. parameters from Medema et al. 1996 were fitted to human volunteer data where 68 adult volunteers where fed doses between 8×10^2 and 1×10^8 cells with milk. The more recent study from Teunis et al. (2005) combined this volunteer study with two milk-related outbreaks. In both outbreaks there was a clear relationship between the observed attack rate and the amount of milk consumed, however the concentration of bacteria in the contaminated milk was not known. Given the difference in r, the choice of model for health-based targets has had ongoing debate. Guidelines in Australia (AGWR, ADWG) and internationally (WHO) have favoured Medema et al. 1996 due to the uncertainty in the outbreak data, although the more infectious model is used in many QMRA studies.
Salmonella	*Salmonella* has not been widely used as a reference pathogen for health-based targets but is particularly relevant for biosolids. A large amount of human volunteer data and outbreak data is available for dose-response modelling. The parameter values of Teunis et al. 2010 reflect the results of a modelling approach that combined data from different sources and recommended the parameters of 8.53×10^{-3} and 3.14 from outbreaks as precautionary values to be used in QMRA.
Norovirus	Given the widespread prevalence of norovirus, and increasing immunity to rotavirus, the use of norovirus as a reference pathogen is increasing. Norovirus cannot be cultured in the laboratory and therefore human volunteer studies are based on molecular data, and various modelling studies have sought to define appropriate parameter values from this data. The parameter values from Messner et al. 2014 have been used in the WHO potable reuse guidelines.
Rotavirus	Human volunteer study of 62 adult men who were administered doses of 9×10^{-3} to 9×10^4 FFU of rotavirus. These data have been widely used for defining health-based targets, although often with different parameter values from the BP approximation.
Adenovirus	While adenovirus has been the preferred reference pathogen for AGWR, the dose-response model previously used was for Rotavirus, given the larger human volunteer dataset. The two parameter values for Adenovirus are from separate modelling studies fitted to a similar dataset (Crabtree et al. 1997 model implemented a subset of the data used by Teunis et al. 2016)
Cryptosporidium parvum (combined)	Model parameters for different species and strains of *Cryptosporidium* have been published. The value of r=0.2 was selected by WHO for application in defining health-based targets and represents the results of a model fitted to data pooled for different strains of *C. parvum.* This value has been used for ADWG and will be used in the revised AGWR.
Cryptosporidium hominis	While data for *C. hominis* is more limited, the results from human volunteer studies do indicate a potentially higher infectivity. This model is used in the Netherlands for regulation of drinking water (Schijven et al., 2014b).
Giardia	The exponential model fitted to *Giardia* data from adult male prison inmates who were administered one of eight doses between 1 and 10^6 cysts, is the only published dose-response model for *Giardia*
Ascaris	There is no human volunteer data for Ascaris. The model that has been used in QMRA was developed based on epidemiological data from Mexico.

Table 6. Probability of illness and DALY weightings

	Probability of illness given infection Pill|inf		DALY per case (DB)	notes
Campylobacter	0.3	(WHO, 2017a)	0.0235	(Gibney et al., 2014)
Salmonella	1	(Teunis et al., 2010)	0.0541	(Gibney et al., 2014)
Norovirus	0.7	(WHO, 2017b)	0.0005	(Gibney et al., 2014)
Rotavirus	0.35	(McBride et al., 2013; WHO, 2017a)	0.0025	(Gibney et al., 2014)
Adenovirus	0.5	(McBride et al., 2013)	0.0025	Assumed to be the same as Rotavirus
C. parvum (combined)	0.7	(WHO, 2017a)	0.0017	(Gibney et al., 2014)
C. hominis	0.7	(WHO, 2017a)		
Giardia	0.45	(McBride et al., 2013)	0.0016	(Gibney et al., 2014)
Ascaris	0.9	(Stevens et al., 2017)	0.000722	(Stevens et al., 2017)

DB: Disease burden in Disability Adjusted Life Years per case

2.5. Risk characterisation

For the risk characterisation, two separate metrics were quantified for each exposure pathway, the critical pathogen concentration and the treatment target (LRV).

The critical pathogen concentration is the concentration of reference pathogen in biosolids at the point of exposure for a particular exposure pathway that equates to an annual health risk of 1×10^{-6} DALY. The critical pathogen concentration ($C_{critical}$) is given by equation 1:

$$C_{critical} = \left(\frac{10^{-6}}{r.P_{ill|inf}.m.f.DB}\right) \text{(Organisms per g biosolids)} \qquad \text{Equation 1}$$

Where *r* is the probability of infection associated with ingestion of one microorganism (Table 4), $P_{ill|inf}$ is the probability of illness given infection (Table 6); *m* is the mass of biosolids or biosolids amended soil in grams ingested/inhaled (Table 3); *f* is the frequency of exposure in events per year (Table 3); and DB is the disease burden per case of illness (Table 6).

The treatment target is defined as the required Log_{10} reduction from raw sludge to exposure to achieve safety (1×10^{-6} DALY pppy). The required Log_{10} reduction is also referred to as the Log Reduction Value (LRV) and is given by equation 2:

$$LRV = Log_{10}\left(\frac{Concentration\ of\ pathogens\ in\ raw\ sludge}{C_{critical} \times solids\ content(\%)}\right) \qquad \text{Equation 2}$$

Where the *concentration of pathogens in raw sludge* refers to the dry weight concentration in raw sludge and *solids content* is the percentage solids of the final biosolid product.

2.6. Achieving the required LRVs for beneficial biosolids reuse

LRVs can be achieved by any process or intervention that leads to a reduction in pathogen exposure including:

- Sludge treatment
- Biosolids treatment
- Environmental controls
- Exposure controls

To achieve safety, the total sum of LRV from treatment and controls must meet or exceed the LRV treatment target.

3. Results

3.1. Quantifying pathogen concentration in sewage, sludge and biosolids

Reported concentrations of reference pathogens in raw sewage are summarised in Table 7. The interpretation of published data relies strongly on the method used for enumeration (see Box 2). Most notably, whether culture or molecular methods have been employed. The following sections outline the selection of default concentrations for NSW sewage.

There is limited data for culturable *Campylobacter* in wastewater. As noted by WHO (WHO, 2017b), the range reported by Soller (2017) is from one study in 1993, and the Australian default value of 7000 organisms per L in the AGWR is more recent and valuable as a default. The estimated mean and upper 95th quantile for *Salmonella* across five sewage plants in Sydney were of 37 000 MPN per L and 170 000 MPN per L respectively. These numbers are considerably higher than the reported international range from Soller (2017) of 3 to 1100 MPN per L, nevertheless the local Sydney Water dataset is much more comprehensive, indicating that a default of 100 000 MPN per L may be reasonable.

Interpretation of the enteric virus data is complicated, given the wide range of reported concentrations and the challenges of enumeration from environmental samples. Concentrations inferred from molecular enumeration approaches lead to much higher reported concentrations (95th up to 1.26×10^9 genome copies per L) than those based on tissue culture (maximum up to 6.93×10^3 infective units per L). The results from the Sydney Water investigation (Appendix A) from ICC-PCR represent a middle ground to these two extremes (estimated mean and upper 95th of 1.1×10^6 and 3.9×10^6) having applied a composite culture/molecular approach (see Box 2). Given the importance of relying on infectious virus units for regulating health risk, there is not sufficient local data to suggest that the current default in the AGWR of 8 000 virus units per L should be changed.

For *Cryptosporidium*, the numbers from Sydney Water treatment plants were lower than expected with an upper 95th percentile (210 oocysts.L^{-1}), which is around an order of magnitude lower than the AGWR default (2000 oocysts.L^{-1}). While King et al. (2017) reported much higher concentrations in Victoria and South Australia (up to 25 675 oocysts per L), they also quantified infectivity, recommending a reduction in concentration by around 0.5 Log_{10} (SA data 0.76 Log_{10} and Vic data 0.42 Log_{10}). The broader south-east Australian data supports the AGWR default of 2000 oocysts per L. For *Giardia*, the numbers in raw sewage are consistently higher than for *Cryptosporidium*, and hence a higher default value of 20 000 cysts per L.

Small populations exhibit greater fluctuations in pathogen concentration in sewage in comparison to large city treatment plants (Barker et al., 2013; Hewitt et al., 2011; Petterson et al., 2016). In the absence of data from smaller plants that may be more representative of regional NSW, results from a modelling study can be considered for comparison (Jahne et al., 2017). For the population size of 100, while *Cryptosporidium* oocysts were only estimated to be present 1.2% of the time, when present the 95th percentile was 1.17×10^6 oocysts per L. When the population increased in size to 1000, the probability of occurrence increased to 11.3% and the 95th percentile of *Cryptosporidium* concentration when present was estimated to be 1.29×10^5 oocysts per L. For enteric viruses with a higher prevalence of excretion, in a population of 1000 the probability of occurrence was 20.3% and the 95th percentile when present was 9.33×10^7 vu per L. The current defaults are based on the assumption of a large population, however further consideration may need to be given to the relevance of these values for smaller plants in regional NSW.

Estimates of reference pathogen concentration in raw sludge based on the partitioning model (Figure 5) are summarised in Table 8. Ascaris was not modelled as it was assumed absent in typical Australian settings and from samples (Irwin et al., 2017), and the recommendation that it should be considered on a case-by-case basis (Deere, 2017). Comparison of the modelled pathogen concentration estimates with reported concentrations in the literature, show that in general, the modelled concentrations are higher, by around an order of magnitude, than results from direct enumeration. There are several explanations for this discrepancy. Firstly, the parameterisation of the model is poorly understood, and improved estimates of partitioning of pathogens to the solids is needed. Furthermore, inactivation of pathogens in primary treatment is not quantified, and is likely to occur to some extent. Nevertheless, reported concentrations are also impacted by uncertainties, and may be underestimates of the true concentration due to the complexity of raw sludge as a matrix for enumeration. This may contribute to poor recoveries, and challenges in culturing organisms from the sludge.

The objective of the QMRA model was to define the treatment requirements (LRV) to achieve safety from the point of raw sludge to end-use. The level of pathogen contamination from which the population should be protected was therefore defined by the modelling results, recognising that they may be an overestimate of concentration in raw sludge. The selected default raw sludge concentrations are summarised in Table 9.

Table 9. Selected reference pathogen concentration defaults in raw sludge for QMRA

Pathogen concentration in raw sludge (microorganisms g.dw^{-1})*					
	Campylobacter	*Salmonella*	Enteric viruses	*Cryptosporidium*	*Giardia*
DEFAULT	2.5×10^4	3.4×10^5	1.6×10^4	6.1×10^3	4.4×10^4

*Modelled for secondary sludge (reported in Table 8)

Table 7. Reference pathogen concentration in raw sewage

	Campylobacter (cfu.L^{-1})	*Salmonella* MPN.L^{-1}	Enteric viruses vu.L^{-1}	*Cryptosporidium* (oocysts.L^{-1})	*Giardia* (cyst.L^{-1})	*Ascaris* (ova.L^{-1})
AGWR defaults	7000		8000	2000		
Sydney Water analysis of raw data (Appendix B) Mean (95th quantile of gamma distribution)		37,000 (170,000)	1.1×10^6 (3.9×10^6)	IFA +ve 51 (210) DAPI +ve 19 (78)	IFA +ve 34,000 (84,000) DAPI+ve: 920 (2,300)	
NSW published literature			2.3×10^7 and 1.5×10^7 genome copies. L^{-1} (Lun et al., 2019)			NA
Australian Literature Range of mean between locations (Range of 95th quantile/percentile between locations)			7.94×10^4– 5.01×10^8 (2.51×10^5– 1.26×10^9) qPCR 1.0×10^3 (2.50×10^3)- 3.2×10^3 (1.00×10^4) MPNIU (Deere and Khan, 2016)	DAPI +ve 212 – 1,591 (619-5469) (Deere and Khan, 2016) IFA +ve Victoria:14- 25 675 (17 877) South Australia: 0 – 6240 (2007) (King et al., 2017)	DAPI +ve 1446 – 16,764 (3,207 – 55,583) (Deere and Khan, 2016)	Not detected in 51, 24 hour composite samples in Victoria (Irwin et al., 2017)
International Literature (Min - Max) (Soller et al., 2017)	900 – 4×10^4 MPN.L^{-1}	3.0 – 1.1×10^3 CFU.L^{-1}	56 – 6.93×10^3 IU.L^{-1}	0.3 – 5×10^4	3.2 – 1×10^4	5 – 670 From endemic areas and NA

Cfu: colony forming units; IFA+ve: immunofluorescence assay; MPN: most probable number; MPNIU: most probable number infectious units; vu: virus units; IU: infectious units

Table 8 Reference pathogen concentration in raw sludge

Estimated pathogen concentration in raw sludge (microorganisms per g dw)					
	Campylobacter	*Salmonella*	Enteric viruses	*Cryptosporidium*	*Giardia*
Sewage concentration point estimates: AGWR defaults (*Campylobacter*, Adenovirus, *Cryptosporidium*) and new proposed defaults (*Salmonella*, *Giardia*) (sludge concentration calculated using fate and transport model as described in text)					
Primary sludge	2.5×10^4	3.3×10^5	1.4×10^4	5.6×10^3	3.7×10^4
Secondary sludge	2.5×10^4	3.4×10^5	1.6×10^4	6.1×10^3	4.4×10^4
Sydney Water (sewage concentration gamma distributed, fitted to raw data. Sludge concentration calculated using Monte Carlo simulation of the fate and transport model as described in text)					
Bondi		2.5×10^4	9.7×10^5	142	5.1×10^4
Mean (95th quantile)		(1.2×10^5)	(3.5×10^6)	(820)	(9.2×10^4)
Liverpool		4.3×10^4	2.2×10^6	110	7.1×10^4
Mean (95th quantile)		(1.8×10^5)	(8.3×10^6)	(410)	(1.7×10^5)
Malabar		1.6×10^6	3.1×10^6	230	3.2×10^4
Mean (95th quantile)		(1.0×10^7)	(1.3×10^7)	(680)	(3.3×10^4)
Rouse Hill		3.4×10^4	3.3×10^6	190	7.5×10^4
Mean (95th quantile)		(1.4×10^5)	(1.5×10^7)	(650)	(1.5×10^5)
Picton		4.4×10^3	1.7×10^6	270	1.7×10^4
Mean (95th quantile)		(1.6×10^4)	(9.3×10^6)	(270)	(3.7×10^4)
LITERATURE COMPARISON					
International Literature (Min - Max) (Brooks et al., 2012)	2.6×10^3 – 3.8×10^3 cfu.g^{-1}	510 - 1.1×10^4 cfu.g^{-1}	28 – 580 pfu.g^{-1}	13 - 64	

dw: dry weight; cfu: colony forming units; pfu: plaque forming units.

3.2. Risk characterisation

The critical pathogen concentrations in biosolids, and performance targets (LRV) required to achieve safety from raw sludge to exposure are summarised in Tables 10 and 11. The very low concentrations required to achieve safety against the 1×10^{-6} DALY benchmark (in the absence of all other controls) means that testing to verify these low levels of pathogens is not practical. The LRVs in Table 11 are intended to capture all treatment and control measures including the use of personal protective equipment and environmental controls.

The lowest critical pathogen concentrations were for viruses followed by *Cryptosporidium* due to their potentially high infectivity in comparison to the bacteria. Highest LRVs were required for *Salmonella* which reflects the high concentrations reported from Sydney Water samples, which were used to estimate the starting concentration in Table 8 and Table 9.

Table 10. Critical pathogen concentrations in biosolids at exposure point (microorganisms. g^{-1} or 1 in *x* g)

Exposure pathway	Exposure group	*Campylobacter*	*Salmonella*	Enteric virus	*Cryptosporidium*	*Giardia*	*Ascaris*
1. Transport: direct exposure during loading and unloading	Transport workers	5.9×10^{-4} (1 in 1684g)	5.5×10^{-4} (1 in 1832g)	3.3×10^{-4} (1 in 3008g)	3.4×10^{-4} (1 in 2975g)	5.6×10^{-3} (1 in 178g)	
2. Composting facility	Handling by workers	5.9×10^{-4} (1 in 1684g)	5.5×10^{-4} (1 in 1832g)	3.3×10^{-4} (1 in 3008g)	3.4×10^{-4} (1 in 2975g)	5.6×10^{-3} (1 in 178g)	
3. Application: application and incorporation	a) Council workers	3.0×10^{-2} (1 in 34g)	2.7×10^{-2} (1 in 37g)	1.7×10^{-3} (1 in 60g)	1.7×10^{-2} (1 in 60g)	2.8×10^{-1} (1 in 4g)	
	b) Farm workers	5.9×10^{-4} (1 in 1684g)	5.5×10^{-4} (1 in 1832g)	3.3×10^{-4} (1 in 3008g)	3.4×10^{-4} (1 in 2975g)	5.6×10^{-3} (1 in 178g)	
	c) Rehabilitation workers	5.9×10^{-4} (1 in 1684g)	5.5×10^{-4} (1 in 1832g)	3.3×10^{-4} (1 in 3008g)	3.4×10^{-4} (1 in 2975g)	5.6×10^{-3} (1 in 178g)	
4. Household garden use	Adults: Incidental	4.5×10^{-2} (1 in 22g)	4.1×10^{-2} (1 in 24g)	2.5×10^{-2} (1 in 40g)	2.5×10^{-2} (1 in 39g)	4.3×10^{-1} (1 in 2g)	
	Children: Incidental	2.2×10^{-2} (1 in 45g)	2.0×10^{-2} (1 in 49g)	1.2×10^{-2} (1 in 81g)	1.3×10^{-2} (1 in 80g)	2.1×10^{-1} (1 in 5g)	
	Children: hand to mouth	7.4×10^{-3} (1 in 135g)	6.8×10^{-3} (1 in 147g)	4.2×10^{-3} (1 in 241g)	4.2×10^{-3} (1 in 238g)	7.0×10^{-2} (1 in 14g)	1.6×10^{-2} (1 in 62g)
5. Public recreation	Adults: Incidental	4.5×10^{-2} (1 in 22g)	4.1×10^{-2} (1 in 24g)	2.5×10^{-2} (1 in 40g)	2.5×10^{-2} (1 in 39g)	4.3×10^{-1} (1 in 2g)	
	Children: Incidental	2.2×10^{-2} (1 in 45g)	2.0×10^{-2} (1 in 49g)	1.2×10^{-2} (1 in 81g)	1.3×10^{-2} (1 in 80g)	2.1×10^{-1} (1 in 5g)	
	Children: hand to mouth	7.4×10^{-3} (1 in 135g)	6.8×10^{-3} (1 in 147g)	4.2×10^{-3} (1 in 241g)	4.2×10^{-3} (1 in 238g)	7.0×10^{-2} (1 in 14g)	1.6×10^{-2} (1 in 62g)
6. Consumption of **food crops**	Consumers	2.5×10^{-2} (1 in 40g)	2.3×10^{-2} (1 in 44g)	1.4×10^{-2} (1 in 72g)	1.4×10^{-2} (1 in 71g)	2.3×10^{-1} (1 in 4g)	5.4×10^{-2} (1 in 19g)
7. Aerosol exposure/inhalation by nearby residents	Residents	7.4×10^{-4} (1 in 135g)	6.8×10^{-3} (1 in 147g)	4.2×10^{-3} (1 in 241g)	4.2×10^{-3} (1 in 238g)	7.0×10^{-2} (1 in 14g)	

*Assuming TSS of 150 $mg.L^{-1}$ in surface water

Table 11. Performance targets (LRV) by exposure pathway from raw sludge to exposure

Exposure pathway	Exposure group	*Campy-lobacter*	*Salmonella*	Enteric virus	*Crypto-sporidium*	*Giardia*
1. Transport: direct exposure during loading and unloading	Transport workers	7.1	8.2	7.1	6.7	6.3
2. Composting facility	Handling by worker	7.1	8.2	7.1	6.7	6.3
3. Application: application and incorporation	a) Council workers	5.4	6.5	5.4	5.0	4.6
	b) Farm workers	7.1	8.2	7.1	6.7	6.3
	c) Rehabilitation workers	7.1	8.2	7.1	6.7	6.3
4. Household garden use	Adults: incidental	5.2	6.4	5.3	4.8	4.5
	Children: incidental	5.5	6.7	5.6	5.1	4.8
	Children: hand to mouth	6.0	7.1	6.0	5.6	5.2
5. Public recreation	Adults: incidental	5.2	6.4	5.3	4.8	4.5
	Children: incidental	5.5	6.7	5.6	5.1	4.8
	Children: hand to mouth	6.0	7.1	6.0	5.6	5.2
6. Consumption of **food crops**	Consumers	5.5	6.6	5.5	5.1	4.7
7. Aerosol exposure/inhalation by nearby residents	Residents	6.0	7.1	6.0	5.6	5.2

*Assuming TSS of 150 mg.L^{-1} in surface water

3.3. Achieving required LRVs

Controls for managing health risk by reducing pathogen concentration and ultimately exposure are summarised in Table 12. Factors influencing the efficacy of each of these controls could be a target for operational monitoring.

Table 12. Control measures for reducing pathogen exposure and key factors influencing efficacy.

	Control	**Factors influencing efficacy**
Sludge Treatment	Mesophilic Anaerobic digestion	Temperature Hydraulic residence time
	Thermophilic anaerobic digestion	Temperature Hydraulic residence time
	Aerobic digestion	Temperature Hydraulic residence time
	Lagoon storage	Temperature Residence time
	Lime treatment	pH, time, temperature
Biosolids Treatment	Composting	Temperature, time
	Storage	Time, temperature, mixing
Environmental Controls	Incorporation to soil	Application rate, mechanism of incorporation
	pH adjustment	Time, pH
	Inactivation	Time, temperature, sunlight
	Crop planting	Application mechanism, Crop type
Exposure	Access restrictions Personal protective equipment	Time, efficacy Usage

A clear understanding of the LRVs that can be achieved by each sludge treatment process, each environmental barrier and each exposure control measure is needed to implement the framework. For biosolids treatment, while information is available regarding the key drivers of inactivation/removal that can be achieved for different pathogen groups, it is not possible to assign LRVs by pathogen group at this time. As identified by Deere et al. (2017) the information is diverse and not linked directly to process conditions and controls (see section 5.15.5 of Deere et al. (2017) including Table 5-11 of that report reproduced from (Sidhu and Toze, 2009)). The subsequent recommendation *6.9 Validation of pathogen inactivation and removal* was drawn from this data gap.

When this framework was applied for the AGWR, the initial values included in the guideline (Appendix B – Table A5.5. and A5.6) were followed up with WaterVal (WaterVal | WATERRA), a national validation program developed to complement the guidelines. There was also an effort to develop a database for validation of different treatment processes, and international data and procedures were identified for common treatment methods. An example of the validation protocol for membrane bioreactors is included in Appendix B. to illustrate how this validation has been implemented in the water reuse context.

Noting the lack of quantitative information available to inform quantification of LRVs, the following example is presented to illustrate the implementation of the approach with hypothetical values.

Table 13. Illustrative example LRVs by control measures for viruses

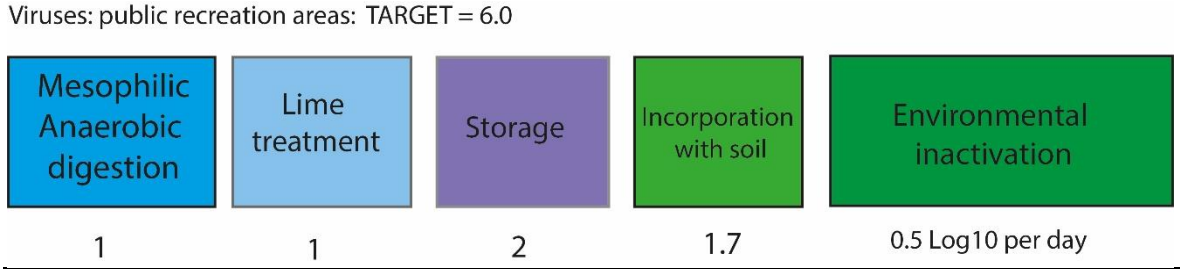
Type of anaerobic digestion	Hypothetical LRV
Mesophilic	1
Lime treatment	1
Storage	2
Thermophilic	3
Composting	6
Incorporation with soil (0.0189 dilution)	1.7
Environmental inactivation	0.5 per day
Access restriction. No public access during application	2
PPE	6

Estimates based on (Eisenberg et al., 2008)

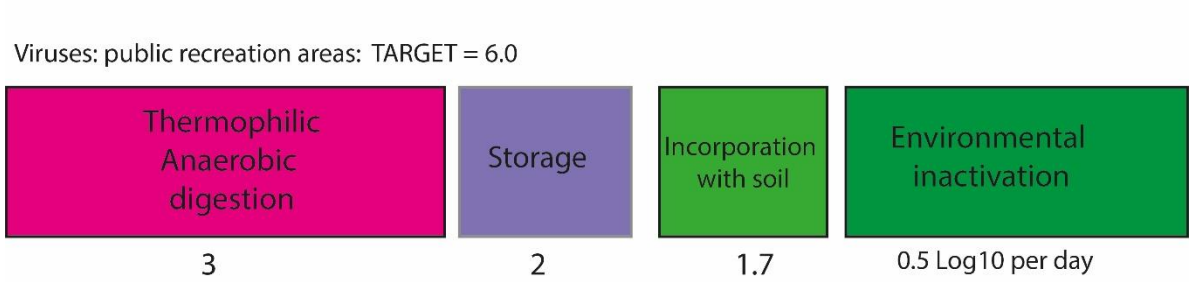
To achieve safety for pathway 5 (public recreation) an overall LRV for viruses of 6.0 is needed to achieve safety for all scenarios (Table 11). Three potential approaches for achieving that LRV are illustrated below.

Once the system is designed to achieve the required LRV, each component should then be monitored according to the factors influencing efficacy in order to verify that the design LRV reduction is being achieved. This validation includes demonstrating quantifiable removal of pathogens under a defined range of conditions and identification of operational monitoring parameters that can be measured to demonstrate ongoing performance in achieving pathogen removal. This assessment needs to be undertaken for each of the pathogen groups including bacteria, protozoa and when relevant helminths

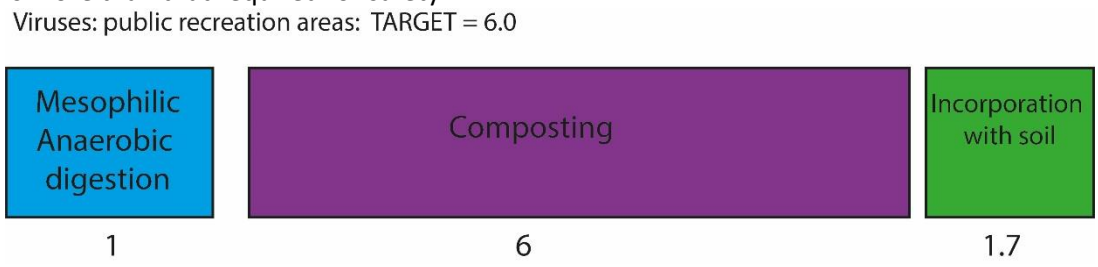
Mesophilic anaerobic digestion, followed by lime treatment, storage, incorporation with soil achieving 5.7 LRV combined. Less than 1 day of inactivation in the environment would be required to achieve overall safety and relying on some access controls over that time would protect public health.



Alternatively, increasing the temperature of digestion to the thermophilic range will increase the LRV to 3; followed by storage and incorporation to achieve at total of 6.7 The LRV is achieved without requiring any access controls.



Thirdly mesophilic digestion followed by composting and incorporation would provide 8.7 LRV which is more than that required for safety.



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4. Conclusions and recommendations

The approach for implementing QMRA for defining health-based performance targets for the safe reuse of biosolids products has been demonstrated. The primary benefits are that a diverse range of end-use practises can be considered, relying on operational verification to ensure public health safety. Combinations of treatment and controls that are specifically relevant to the local context can be implemented and managed to achieve safety. The resulting framework is therefore intended to be flexible, with biosolids products tailored to be fit for purpose. The objective is to ensure public health safety, while at the same time preventing over-design of treatment, or unnecessarily restrictive controls. The following conclusions and recommendations are drawn from this study.

1. Uncertainty and sensitivity analysis of the current model

The quantitative treatment targets presented in this report should be considered a first pass for illustration of the approach. Further work is needed to refine the results including to:

- assess the importance of locally relevant values for pathogen concentration in biosolids, especially for small and regional communities
- review the fate and transport of pathogens during wastewater and sludge treatment including the parameters used in the model for quantifying pathogen concentration in raw sludge
- assess the importance of the magnitude and frequency of exposure to biosolids
- assess the importance of model defaults including dose-response models and point estimates of probability of illness

Recommendation 1: Undertake a sensitivity analysis of the current model to assess the importance of uncertainties in model inputs and identify any critical data gaps.

2. Quantify pathogen concentration in sludge and biosolids

Quantifying the concentration of appropriate reference pathogens in raw and treated sewage sludge is a considerable challenge and data of relevance to NSW (both city and regional) is limited. Recent data from Sydney Water summarised in this report illustrates the variability in concentration for different pathogens. Importantly, the high concentration of human enteric viruses, not only in raw sludge but also potentially in biosolids products, needs further investigation. While some pathogens may be identified by analytical methods, some methods are unable to indicate whether those organisms are in an infectious state. In defining health-based treatment targets consideration must focus on infectious pathogens and seek to eliminate from the calculation those microorganisms that have been inactivated.

Recommendation 2: Support and (when possible) initiate targeted data collection programs that focus on quantifying the magnitude and variability of infectious pathogens in sewage, raw sludge and biosolids products for all of NSW.

3. Quantify pathogen LRVs for sludge and biosolids treatment processes

The performance of various sludge and biosolids treatment processes for inactivation of pathogens is poorly understood. Linking achievable LRVs to measurable process conditions

such as residence time and temperature, is essential for the practical implementation of the framework, however this data currently does not exist.

Recommendation 3a: Undertake a targeted and quantitative literature review of published LRVs for pathogens and indicators for all processes and controls within the scope of the proposed guidelines. LRVs should be directly linked to measurable process variables from the reviewed publication.

Recommendation 3b: Support and (when possible) initiate targeted data collection programs that focus on quantifying the LRVs of sludge and biosolids treatment processes. Such programs should focus on pathogen inactivation mechanisms and link reduction to measurable process variables.

4. Continue to consult on the scope of exposure pathways

The exposure pathways quantified in this project were identified by stakeholders that participated in problem formulation. Further work is needed to ensure that the scope of exposures is appropriate to the NSW context, including any vulnerable groups that may need consideration.

Recommendation 4: Continue consultation as broadly as possible with respect to exposure pathways to be considered in any future guideline. Consideration should be given to the needs of any vulnerable groups, especially first nations peoples.

5. Develop a roadmap to industry implementation

The same framework has been applied for the safe reuse of wastewater in Australia since 2006. The implementation in the water sector required a significant shift in mindset from relying on end-point sampling controls for protection of public health, to overall system management. While end-point controls were known to be inadequate and restrictive for supporting wider wastewater reuse applications, moving to an alternative framework took time for the industry to embrace. The shift in approach required a change in focus for the treatment plant operator; change in targets for the regulator; and a change in expectations of the end-users/ customers. The result has been an industry more aware of their responsibility to manage process performance, identify and manage system failures, and increased end-user confidence in consistent product safety. At the same time, applications of wastewater reuse across the country have increased, strengthened, and broadened in scope.

Recommendation 5: Develop a roadmap to implementation in consultation with the wastewater industry. Identify all the key industry engagement tasks and supporting documentation required to achieve successful implementation. Undertaking this process in collaboration with the water industry will enable NSW EPA to benefit from and build on the learnings of this closely aligned sector.

Appendix A. Quantitative Microbial Risk Assessment: Application for Water Safety Management. Case Study 5.

Appendix B. WaterSecure 2017, Membrane bio-reactor, WaterVal validation protocol, Australian WaterSecure Innovations Ltd, Brisbane.

Appendix C. Occurrence of indicators and pathogens in sewage and biosolids at five resource recovery plants in Sydney, Australia.

Sewage and biosolid products were sampled from five sewage treatment plants in Sydney, Australia. Samples were analysed for pathogens and indicators, and the reduction across each of the five sludge treatment trains was evaluated. The data from this study were made available by Sydney Water Corporation to support the work of the NSW EPA.

Sampling and analyses

Five resource recovery plants were selected for the study representing different population sizes, and sludge treatment processes. Samples were collected between July 2013 and June 2014 from both raw sewage (n=44) and biosolids products (n=60). Sampling regimes are summarised in Table A.1. Samples were analysed for microbial indicators (*E. coli* and *Enterococci*) and pathogens (*Salmonella*, Adenovirus, *Cryptosporidium* and *Giardia*)
For *Salmonella* the method consisted of presence/ absence of culture on specific media, with three replicates at three serial dilutions; for Adenovirus a combined tissue culture, PCR method was applied to assess the presence/absence from five replicates at four serial dilutions. For *Cryptosporidium* and *Giardia* the number of immunofluorescence assay (IFA) positive oocysts, and the number of DAPI (4',6-diamidino-2-phenylindol) positive oocysts were counted. All virus and protozoa analysis included internal recovery controls.

Table A.1. Monitoring program for sewage and biosolids at Sydney Water treatment plants.

Plant	Design capacity (PE)	Raw sewage samples (n)	Sewage Treatment	Sludge Treatment	Biosolids Products (n)
Bondi	1 106 185	16	Primary sedimentation	Anaerobic digestion	16
Liverpool	58 894	16	Primary sedimentation and Activated Sludge	Anaerobic digestion	16
Malabar	1 482 500	4	Primary sedimentation	Anaerobic digestion	4
Rouse Hill	48 824	4	Intermittently decanted aerated lagoon (IDAL)	Anaerobic digestion	12
Picton	13 288	4	Intermittently decanted aerated lagoon (IDAL)	Lagoon	12
TOTAL		44			60

PE: population equivalents

Data analysis: quantifying concentration

A gamma distribution (shape, scale) was fitted to the microbial datasets using the method of maximum likelihood. The gamma distribution was selected as a flexible parametric distribution that has been widely applied for quantifying pathogen concentration in

environmental media. Distributions were fitted to site specific datasets and pooled datasets for each pathogen.

Indicator data: Gamma distributions were fitted directly to the reported concentrations of *E. coli* and enterococci.

Salmonella: The Poisson distribution was used to find the maximum likelihood bacteria concentration for each combination of positive/negative results. Less than detection limit were replaced with the detection limit. Greater than values were replaced with the maximum concentration. The Gamma distribution was fitted to the sample specific Poisson concentration estimates.

Adenovirus: The Poisson distribution was used to find the maximum likelihood virus concentration for each combination of positive/negative results, and then corrected for method recovery. Method recovery was quantified as the maximum likelihood estimate of the number recovery divided by the spike. Results were corrected for volume and sample mass to estimate virus concentration per litre of raw sewage and per gram dry weight of biosolids. A gamma distribution was fitted to each dataset from each treatment plant.

Cryptosporidium: For *Cryptosporidium* counts, the negative binomial distribution was fitted to the raw counts (n) paired with their recovery and sample volume/mass:

$$g(n|\lambda,\rho,V,\pi)=\frac{\Gamma(\rho+n)}{n!\Gamma(\rho)}\frac{\lambda^{\rho}(\pi V)^{n}}{(\lambda+\pi V)^{\rho+n}}$$

Assuming this model, the log-likelihood function was constructed for each dataset of counts (n_i), volumes (V_i) or mass (m_i) and paired recoveries (π_i=recovery/100), and optimised to find the maximum likelihood estimator (MLE) of λ and ρ.

The probability that an oocyst was DAPI positive was assumed to be a binomial process, with probability of DAPI positive given by *p*. The probability of identifying *x* DAPI positive oocysts, given a total of *n* oocysts examined was given by:

$$f(x|n,p)=(1-p)^{n-x}p^{x}\binom{n}{x}$$

Giardia: For *Giardia* counts, the negative binomial distribution was fitted to the raw counts (n) paired with their recovery and sample volume/mass:

$$g(n|\lambda,\rho,V,\pi)=\frac{\Gamma(\rho+n)}{n!\Gamma(\rho)}\frac{\lambda^{\rho}(\pi V)^{n}}{(\lambda+\pi V)^{\rho+n}}$$

Assuming this model, the log-likelihood function was constructed for each dataset of counts (n_i), volumes (V_i) or mass (m_i) and paired recoveries (π_i=recovery/100) and optimised to find the maximum likelihood estimator (MLE) of λ and ρ (gamma parameters).

The probability that a cyst was DAPI positive was assumed to be a binomial process, with probability of DAPI positive given by *p*. The probability of identifying *x* DAPI positive cysts, given a total of *n* cysts examined was given by:

$$f(x|n,p)=(1-p)^{n-x}p^{x}\binom{n}{x}$$

Results

Microbial indicators

The mean and 95th quantiles of the gamma distributions fitted to the E. coli and enterococci data are summarised in Table A.3. The uncertainty around the estimated concentrations is higher for smaller datasets. For example, the concentration of *E. coli* in biosolids from Malabar is notably higher than other products, however only 4 samples were taken, and one sample reported a particularly high concentration of 1.9×10^{10} cfu.100g wet weight. The upper 95th quantile of the gamma distribution fitted to these counts reflects this high value at 1.91×10^{9} cfu.g dry weight.

Table A.3. Mean and 95th quantile of the maximum likelihood gamma distributions for *Enterococci* and *E. coli*

		Sewage (cfu.L^{-1})			Biosolids (cfu.g dry w^{-1})		
		n	Mean	Upper 95 Percentile	n	Mean	Upper 95 Percentile
Bondi	Enterococci	16	1.7×10^{7}	2.7×10^{7}	16	2.8×10^{4}	8.7×10^{4}
	E. coli		1.5×10^{8}	3.2×10^{8}		9.1×10^{5}	3.3×10^{6}
Liverpool	Enterococci	16	5.3×10^{7}	2.2×10^{8}	16	4.5×10^{6}	3.0×10^{7}
	E. coli		2.9×10^{8}	6.9×10^{8}		1.3×10^{7}	7.4×10^{7}
Malabar	Enterococci	4	2.6×10^{6}	8.6×10^{6}	4	1.3×10^{5}	3.2×10^{5}
	E. coli		1.6×10^{8}	4.5×10^{8}		2.0×10^{8}	1.1×10^{9}
Rouse Hill	Enterococci	4	5.0×10^{7}	1.7×10^{8}	12	3.0×10^{7}	1.2×10^{8}
	E. coli		2.4×10^{8}	4.4×10^{8}		7.4×10^{5}	2.0×10^{6}
Picton	Enterococci	4	1.2×10^{7}	2.1×10^{7}	12	2.9×10^{5}	2.0×10^{6}
	E. coli		1.1×10^{8}	2.2×10^{8}		1.4×10^{5}	8.4×10^{5}

The mean and upper 95th quantiles of the gamma distribution fitted to *Salmonella* presence absence results and Adenovirus presence absence results corrected for recovery, are summarised in Table A.4. The mean and upper 95th quantiles of the gamma distribution fitted to the *Cryptosporidium* and *Giardia* IFA +ve counts are summarised in Table A.5. The estimated mean concentration of *Salmonella* in sewage across all sites was 37 000 MPN.L^{-1}, with an upper 95th quantile of 170 000MPN.L^{-1}. The estimated mean concentration of Adenovirus in sewage was 1.1×10^{6} MPN.L^{-1} (3.9×10^{6}), 51 (210). The gamma distribution fitted to the pooled sewage concentration data are illustrated in Figures A1 to A4.

Table A.4. Mean and 95th quantile of the maximum likelihood gamma distributions for *Salmonella* and Adenovirus

Salmonella

	Sewage (MPN.L^{-1})		Biosolids (MPN.g dry w^{-1})	
	Mean	Upper 95 Percentile	Mean	Upper 95 Percentile
Bondi	8.3×10^3	4.1×10^4	5.8×10^2	3.7×10^3
Liverpool	1.9×10^4	7.8×10^4	1.2×10^3	5.9×10^3
Malabar	2.9×10^5	1.9×10^6	4.6×10^2	2.7×10^3
Rouse Hill	1.0×10^4	4.2×10^4	8.9×10^1	3.3×10^2
Picton	2.1×10^3	8.0×10^3	1.8×10^2	8.3×10^2
Pooled data	3.7×10^4	1.7×10^5		

Adenovirus

	Sewage (MPN.L^{-1})		Biosolids (MPN.g dry w^{-1})	
	Mean	Upper 95 Percentile	Mean	Upper 95 Percentile
Bondi	6.0×10^5	2.2×10^6	4.3×10^2	1.4×10^3
Liverpool	1.6×10^6	5.9×10^6	7.3×10^3	2.4×10^4
Malabar	1.1×10^6	4.6×10^6	1.3×10^3	2.9×10^3
Rouse Hill	1.7×10^6	7.8×10^6	6.6×10^3	1.8×10^4
Picton	1.4×10^6	7.7×10^6	1.3×10^4	4.0×10^4
Pooled data	1.1×10^6	3.9×10^6		

Table A.5. Mean and 95th quantile of the maximum likelihood gamma distributions for *Cryptosporidium* and *Giardia*

Cryptosporidium

	Sewage (oocysts.L^{-1})			Biosolids (oocysts.g dry w^{-1})		
	Mean (IFA +ve)	Upper 95 Percentile (IFA +ve)	Probability DAPI Positive	Mean	Upper 95 Percentile	Probability DAPI positive
Bondi	53	310	0.45	11	29	0.16
Liverpool	55	195	0.21	70	300	0.33
Malabar	51	148	0.46	130	410	0.24
Rouse Hill	64	223	0.46	96	450	0.67
Picton	15	15	< 0.14	320	1463	0.58
Pooled data	51	210	0.37			

Giardia

	Sewage (cyst.L^{-1})			Biosolids (cysts.g dry w^{-1})		
	Mean (IFA +ve)	Upper 95 Percentile (IFA +ve)	Probability DAPI positive	Mean	Upper 95 Percentile	Probability DAPI positive
Bondi	3.1×10^4	5.7×10^4	0.023	6.1×10^4	1.7×10^5	1.3×10^{-3}
Liverpool	4.7×10^4	1.1×10^5	0.018	3.4×10^5	9.2×10^5	6.3×10^{-4}
Malabar	1.1×10^4	1.2×10^4	0.038	2.1×10^5	6.0×10^5	2.2×10^{-3}
Rouse Hill	3.6×10^4	7.0×10^4	0.078	1.6×10^4	7.3×10^4	$< 8.3\times 10^{-4}$
Picton	1.3×10^4	2.8×10^4	0.050	1.4×10^4	3.1×10^4	2.5×10^{-3}
Pooled data	3.4×10^4	8.4×10^4	0.027			

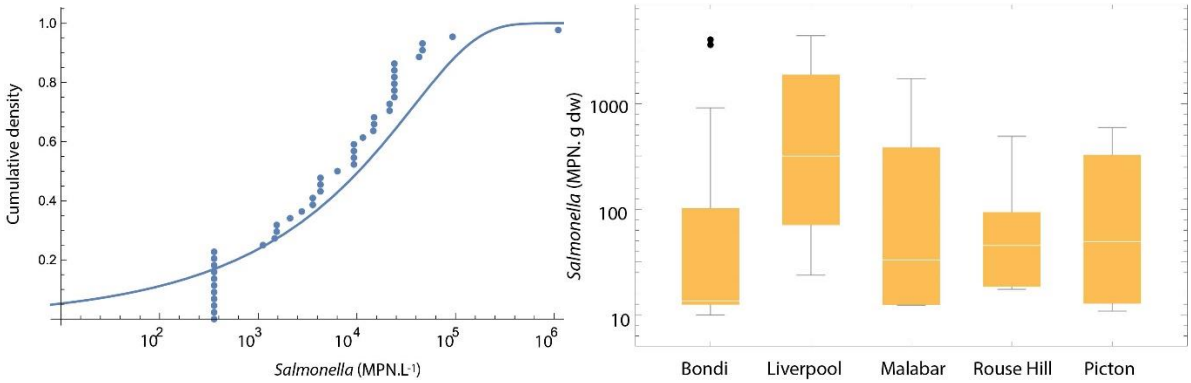

Figure A.1. Left: Cumulative density function for *Salmonella* concentration in raw sewage. Maximum likelihood gamma distribution (solid line) fitted to individual sample concentration estimates (dots) from all five treatment plants. Right: Box Whisker plot of estimated *Salmonella* concentrations (MPN.g^{-1} dry weight) in final biosolids products from 5 wastewater treatment plants

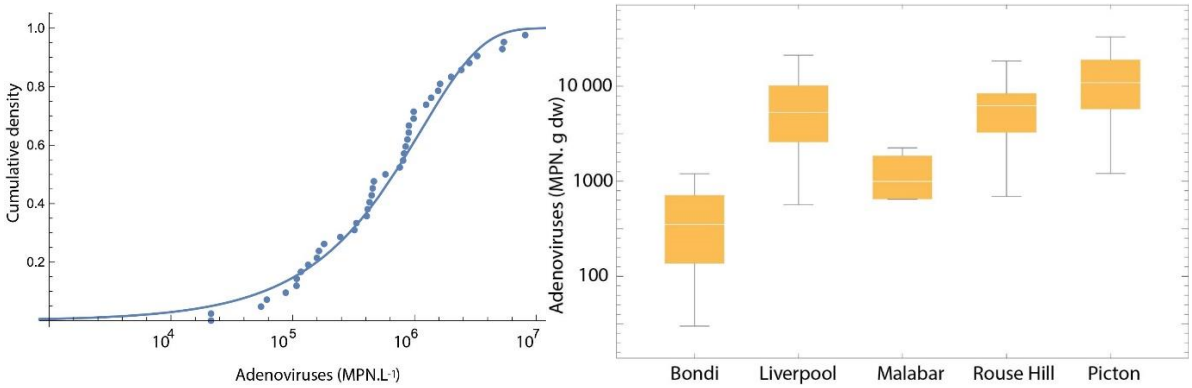

Figure A.2. Left: Cumulative density function for Adenovirus concentration in raw sewage. Maximum likelihood gamma distribution (solid line) fitted to individual sample concentration estimates (dots) from all five treatment plants. Right: Box Whisker plot of estimated Adenovirus concentrations (MPN.g^{-1} dry weight) in final biosolids products from 5 wastewater treatment plants

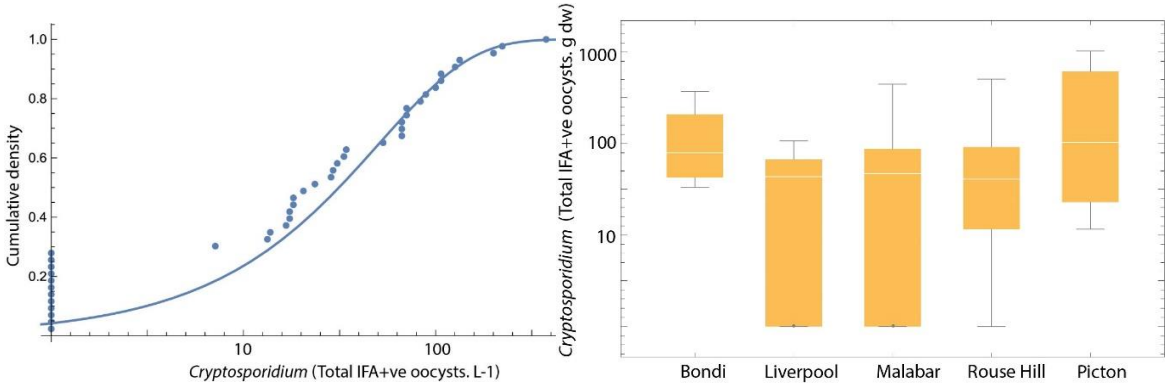

Figure A.3. Left: Cumulative density function for *Cryptosporidium* (Total) concentration in raw sewage. Maximum likelihood gamma distribution (solid line) fitted to individual sample concentration estimates (dots) from all five treatment plants. Right: Box Whisker plot of estimated *Cryptosporidium* (Total) concentrations in final biosolids products from 5 wastewater treatment plants

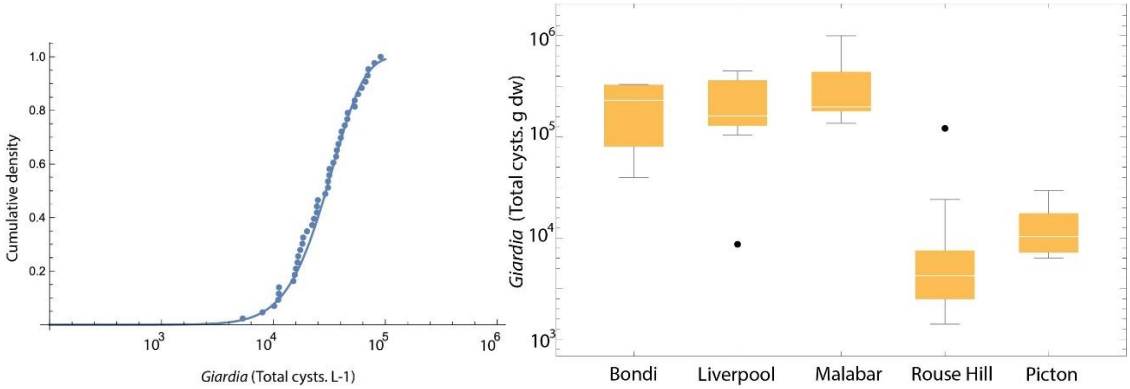

Figure A.4. Left: Cumulative density function for *Giardia* (Total) concentration in raw sewage. Maximum likelihood gamma distribution (solid line) fitted to individual sample concentration estimates (dots) from all five treatment plants. Right: Box Whisker plot of estimated *Giardia* (Total) concentrations in final biosolids products from 5 wastewater treatment plants

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