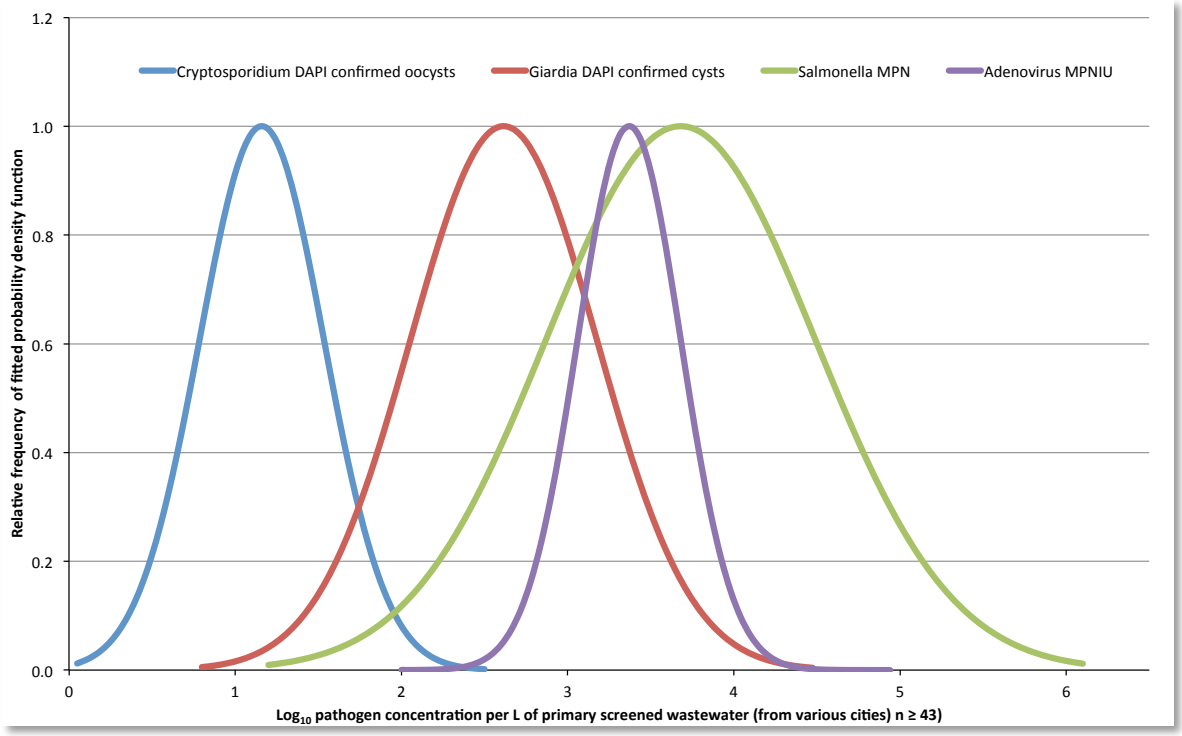
Australian Water Recycling
Centre of Excellence

Milestone Report

National Validation Framework for Water Treatment Technologies

Collation and Analysis of Source Water Pathogen Monitoring Data

Water Futures and University of NSW
September 2016



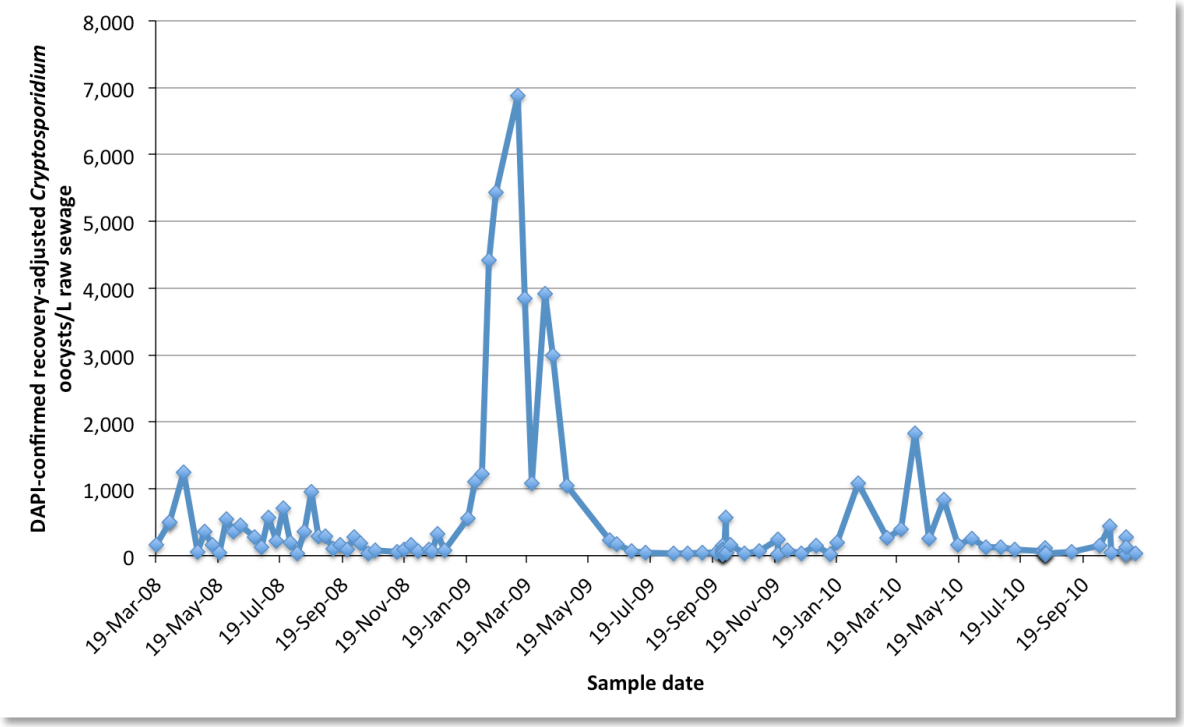


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Table of Contents

Table of Figures

Table of Tables

1 Executive summary

1.1 Purpose of this study

The principal aim of this milestone, (being Milestone 3 of Sub-project 4 of NatVal Stage 2.2, Phase 2), was the establishment of appropriate source water pathogen concentrations for sewage as a recycled water source. The information needs to be in a form suitable for updating the Australian Guidelines for Water Recycling (AGWR, NRMMC *et al*., 2006, 2008 and 2009) as well as to provide an input into the core components of Sub-project 4. The concentrations were to be expressed in terms of both simple summary statistics, such as percentiles, as well as probability density functions that capture the pattern of variability in those concentrations. A secondary aim was to develop and outline a recommended approach for characterising pathogen concentrations in other recycled water sources.

1.2 Study scope

The principal source of recycled water in Australia, and the emphasis of the current AGWR, is raw municipal sewage (untreated, screened or primary settled). Therefore, the focus of this project has been on reviewing and analysing recent data from raw municipal sewage. In addition, sufficient data was provided for secondary treated municipal sewage (clarified activated sludge) that this was included in this analysis even though not anticipated during project inception. Good quality recent pathogen monitoring data on urban stormwater and grey water have not been forthcoming beyond that used to support the current AGWR series.

1.3 Principal study findings

1.3.1 Protozoa

Whilst consistently outnumbered by *Giardia* spp. cysts in raw sewage, due to its greater transmissibility and persistence than most protozoan pathogens, *Cryptosporidium* spp. oocysts were selected in developing the AGWR as the reference pathogen for assessing and managing risks from protozoa (NRMMC *et al*., 2006). The broad body of data was consistent with the current AGWR default summary statistic for *Cryptosporidium* of a 95%ile of 2,000 oocysts per L in raw municipal sewage. The evidence from extensive monitoring since 2006 has not provided any evidence that this statistic should be altered. Concentrations of *Giardia* remain consistently approximately one order of magnitude higher than *Cryptosporidium*. Whilst the greater persistence and transmissibility of *Cryptosporidium* means that its role as the reference pathogen for protozoa remains justified for well-treated effluent, it is important not to overlook *Giardia* in situations where there is a minimal level of treatment and where exposure controls represent the principal means of protection against transmission.

1.3.2 Viruses

Due to its more numerous occurrence than most viral pathogens, its double-stranded DNA genome (being more stable than the RNA genomes of most human pathogenic waterborne viruses) and the ability to be detected using culture-based methods directly from raw sewage, adenovirus was selected in developing the AGWR as the reference pathogen for assessing and managing risks from viruses (NRMMC *et al*., 2006). The recent data confirm that adenovirus is consistently and readily isolated from sewage using culture-based methods. In addition, the viruses are readily monitored using molecular methods. Adenovirus is the most numerous virus enumerated in raw municipal sewage, supporting its selection as the reference virus for estimating viral concentrations in that matrix. Where the monitoring methods used since 2006 have remained approximately the same as those used prior to 2006, the more recent data was consistent with the historical data form which the AGWR default summary statistic for viruses of 8,000 virions per L in raw municipal sewage was derived. The evidence derived from cultivation-based methods from extensive monitoring since 2006 has not provided any evidence that this statistic needs to be altered. More recent molecular assays, particularly those that better correct for methodological recovery efficiency, report higher concentrations than traditional methods by a margin of two to three $\log_{10}$. However, these molecular methods are not measuring the same viral properties as the traditional methods that form the basis of the dose-response models that underpin the health-based targets. There is evidence that the novel methods enumerate viral constituents rather than being representative of the concentration of viruses that would be infectious *in vivo*. Therefore, there is no evidence that the AGWR default summary statistic for infectious viruses needs to be adjusted. Nonetheless, ongoing research to better understand viral concentrations in sewage are warranted with particular attention being given to the

importance of correcting for methodological recovery efficiencies and using assays that can be interpreted with respect to infectious virus concentrations.

1.3.3 Bacteria

Due to its greater transmissibility than most bacterial pathogens, *Campylobacter* was selected in developing the AGWR as the reference pathogen for assessing and managing risks from bacteria (NRMMC *et al*., 2006). However, due to its ability to amplify outside of the host and its inclusion in biosolids guidelines, more recent studies have monitored *Salmonella*. The more recent data imply that that the default *Campylobacter* concentration given in the AGWR of 7,000 bacteria per L might fail to ensure compliance with the health-based target for *Salmonella,* with the concentration of total non-typhoid *Salmonella* spp. (approximately 200,000 bacteria per L) being approximately 1.5 $\log_{10}$ greater than the default *Campylobacter* concentration given in the AGWR. In practice, because protozoan and viral pathogen reduction overwhelmingly dominate treatment requirements there is no urgency in relation to this finding, which is somewhat academic. Nonetheless, utilising the concentration of total non-typhoid *Salmonella* spp. as the default starting concentration for bacteria in sewage is defensible based on this more recent data.

1.3.4 Variability

Taken at face value, the influence of variables such as i) city, ii) sewage treatment plant (STP) within a city, and iii) sampling location within a process step (e.g. screened versus primary effluent), appeared to have a very significant effect on pathogen concentrations. However, in some cases it seems more mechanistically plausible that these differences arise from sampling and analytical variables and not true differences in underlying pathogen concentrations. It is not likely that large STPs within integrated cities have consistently and markedly different concentrations of common pathogens during the same time period. However, some variations, such as seasonal *Cryptosporidium* peaks, appeared to be real phenomena. Within any particular dataset for a specific sampling location, the variability was small enough that from a health risk management perspective the seasonal and other peaks can be adequately accommodated with the use of the 95%ile statistic as a default value as was the case with the current version of the AGWR. This use of a 95%ile default statistic provides some conservatism and appears to be an appropriate statistic to use. With the presence of substantial additional data since 2006, it could be argued that such conservatism is no longer required. The data were found to fit well to lognormal distributions, (but not to normal or Poisson distributions), so that the arithmetic mean would arguably be a more representative but still slightly conservative summary statistic. It is noted that the arithmetic mean is the summary statistic selected for the health-based targets program for drinking water. However, across the board, the arithmetic mean was only between 0.3 and 0.6 (median and mean 0.5) $\log_{10}$ less conservative than the 95%ile so that selecting between these two statistics would only make a marginal difference to treatment requirements.

1.4 Recommendations

At present the evidence provided since 2006 does not point to the need to change the AGWR default pathogen concentrations used for risk assessment and risk management planning for recycled water schemes for viruses and protozoa. However, the role of wholly molecular methods, or culture-based methods with molecular confirmation steps, needs to be carefully considered in future monitoring programs, particularly for viruses. The assay used to assess pathogen concentrations in water needs to be reported, with results possibly needing to be transformed by orders of magnitude if molecular assays do not measure infectivity in order to match the methods used in assaying pathogen concentrations for dose-response analyses. For bacteria, a move to *Salmonella* in place of *Campylobacter* is possibly warranted but such a change is unlikely to have any material effect on treatment processes. Furthermore, in future assays of viruses, the inclusion of controls relating to method recovery is recommended as is now routine for assays of protozoan pathogens.

1.5 Acknowledgements

For this project, three leading-edge public water utilities, Melbourne Water, SA Water and Sydney Water, generously provided extensive data and information. The efforts of key staff in gaining approval to provide data to support the project, and their trust placed in the project participants in utilising their data, is gratefully acknowledged and appreciated. Without such strong support and effort by these utilities and their staff this project would not have been possible. Detailed and important advice and support in relation to the interpretation of data was received from the UNSW School of Biotechnology and Biomolecular Sciences (Professor Peter White and Ms Jennifer Lun) as well as SA Health (Dr David Cunliffe).

2 Acronyms and abbreviations

Term	Definition
ADWG	Australian Drinking Water Guidelines (2011)
AFRI	Acute Febrile Respiratory Illness
AGWR	Australian Guidelines for Water Recycling (NRMMC *et al.*, 2006, 2008 and 2009)
ALS	Australian Laboratory Services
AS/NZS	Standards Australia/Standards New Zealand
AWQC	Australian Water Quality Centre
AWRCoE	Australian Water Recycling Centre of Excellence
BOD	Biological Oxygen Demand
CCPCR	Cell Culture Polymerase Chain Reaction
C•T	Disinfectant dose; the product of disinfectant concentration post contact time (mg/L) and estimated contact time (min)
DALY	Disability-adjusted Life Year
DAPI	4'6-diamidino-2-phenylindole
DIC	Differential interference contrast
EP	Equivalent persons
EPHC	Environmental Protection Heritage Council
GI	Gastrointestinal Illness
HBT	Health-based target
HCGI	Highly Credible Gastrointestinal Illness
LRV	Log Reduction Value
MPN	Most Probable Number Infectious Units
NATA	National Association of Testing Authorities
NatVal	National Validation Framework for Water Recycling
NHMRC	National Health and Medical Research Council
NRMMC	Natural Resources Management Ministerial Council
NTU	Nephelometric Turbidity Units

Term	Definition
qPCR	Quantitative Polymerase Chain Reaction
SBR	Sequencing Batch Reactor
SCADA	Supervisory Control And Data Acquisition
STP	Sewage Treatment Plant
TSS	Total Suspended Solids
US	United States
USEPA	United Stated Environmental Protection Agency
UV	Ultraviolet
WaterRA	Water Research Australia Limited
WHO	World Health Organization
WWTP	Wastewater Treatment Plant

3 Background

3.1 Introduction

Water Research Australia Limited (WaterRA) was engaged by the Australian Water Recycling Centre of Excellence (AWRCoE) under its National Validation Framework for Water Recycling (NatVal) to conduct NatVal Stage 2.2, Phase 2: "*High Priority Research and Development Gaps*". WaterRA has structured that research into five Sub-projects under Program Manager Dr Cedric Robillot.

Sub-project 4 is entitled "Multiple Barriers (Integrated Systems)" and is being conducted under the direction of Sub-project Leader A/Prof Stuart Khan of UNSW.

Milestone 3 under Sub-project 4 is entitled: "Collation and analysis of source water pathogen monitoring data". This milestone is critical to the whole NatVal program in that it establishes the best-supported estimate of pathogen concentrations in recycled water sources (e.g. sewage, stormwater and greywater). This document summarises Milestone 3.

3.2 Objectives

The principal aim of the Milestone 3 project was the establishment of appropriate source water pathogen concentrations for common recycled source water categories in a form suitable for updating the Australian Guidelines for Water Recycling (AGWR; NRMMC *et al*., 2006, 2008 and 2009) and for input into the remainder of Sub-project 4. The concentrations needed to be expressed in terms of both simple summary statistics, such as 95th percentiles, as well as probability density functions (to capture the pattern variation). A secondary aim was to develop and outline a recommended approach for characterising pathogen concentrations in other recycled water source types on a context-specific basis. A report on source water pathogen characterisation was to be provided as a project deliverable (this report).

3.3 Scope

For most Australian recycled water schemes it is the default pathogen concentrations given in the AGWR for raw municipal sewage form the start point for validation of recycled water schemes. Consistent with this principal source of recycled water in Australia, and the emphasis of the current AGWR, the focus of this project has been on reviewing and analysing updated data from raw municipal sewage (untreated, screened or primary settled).

Fortuitously sufficient data has been provided for secondary (activated sludge plant) treated municipal sewage that this was included in this analysis even though not anticipated during project inception. A number of recycled water schemes have utilised secondary treated municipal sewage as their start point for validation. However, the results are less generally applicable since different secondary treatment processes vary significantly in terms of the pathogen concentrations that they yield.

At the time of writing, good quality new pathogen monitoring data, (beyond that used to support the current versions of the AGWR), on urban stormwater and grey water have not been forthcoming.

3.4 Acknowledgements

Two leading-edge public water utilities, Melbourne Water and SA Water, generously provided much of the raw and secondary treated effluent data that was utilised to underpin the first edition of the AGWR (2006). Considerably improved data has been collated since that time. For this project, three leading-edge public water utilities, Melbourne Water, SA Water and Sydney Water, generously provided updated data and information.

Under the NatVal Project Agreements, all project participants are committed to confidentiality in relation to the data. The confidential data that has been generously provided to pathogen monitoring data cannot be shared or identified. No specific datasets are to be identified in any publicly available reporting material. The data has been collated and utilised along with other data to provide an Australian national position on pathogen concentrations and the variation thereof as part of updating the national guidelines.

It should be noted that selective use of the data meant that some data was not used where data of higher quality was found that yielded statistically significantly different results. However, as part of maintaining confidentiality, no indication is given of which data was used.

4 Current recycled water guidelines

Default pathogen concentrations form the treatment requirements in the AGWR for recycled water sources. The AGWR proposed that since pathogen concentrations vary over a wide range, 95th percentiles should be used for determining these default pathogen log reduction values. Data were collected from a number of pre-existing or specially collected sampling programs. For the sewage recycling guidelines, leading edge water utilities in many cases already had pathogen monitoring data and they kindly provided that data to support the development of the AGWR. For the stormwater guidelines, a special monitoring program was carried out to characterise pathogen concentrations in urban stormwater catchments. Data from leading health authorities was used to inform the estimates for roofwater. The concentrations derived from those guidelines are summarised below and in Table 4-1.

4.1.1 Sewage

Analyses from two Australian sewage treatment plants were used to provide the data that set the basis of pathogen reduction requirements for recycled water that started with sewage as its source. The data was sourced via SA Department of Health from SA Water and from Melbourne Water. The concentrations were set at 2,000 protozoa, 8,000 viruses and 7,000 bacteria per L of raw sewage and these were considered to represent 95th percentiles. These values were considered to be consistent with international data and suitable as default values in determining treatment and/or exposure pathogen reduction performance targets that in turn needed to be validated. In addition, the AGWR allowed for system-specific data on pathogen concentrations to used as an alternative to the default values.

4.1.2 Greywater

For greywater, the concentrations of pathogens were assumed to be the same as for sewage but reduced by the extent of measured *E. coli* concentrations. For instance, assuming 10^7 *E. coli* per 100 ml in raw sewage then if greywater contains 10^5 *E. coli* per 100 mL (a fairly typical value for greywater) it can be assumed to have the equivalent of 1% sewage. Therefore, the pathogen reductions required for sewage are simply reduced by a magnitude of 2 $\log_{10}$. In such a case the concentrations would be 20 protozoa, 80 viruses and 70 bacteria per L of greywater and these were considered to represent 95th percentiles. Similarly, if greywater contained 10^4 *E. coli* per 100 mL the reduction required would be reduced by a magnitude of 3 $\log_{10}$.

4.1.3 Stormwater

For the AGWR stormwater guidelines, analyses from four sites in Sydney, from a special study undertaken to support the AGWR development and funded by NSW EPA, were used to represent sewered residential area urban stormwater. The data were collected specifically to support the development of the guidelines. Microbial urban stormwater quality summary statistics were derived from 59 samples covering three sites with relatively high sewer overflows and capturing 11 dry weather and 48 wet weather samples. The wet weather samples were collected from four storm events for each of the four sites, with sampling during the early, mid and late hydrograph stages. The default pathogen concentrations derived were 1.8 protozoa, 1 virus and 15 bacteria per L of raw stormwater and as 95th percentiles. The data were considered to be representative of stormwater quality through the range of conditions within which water would be harvested.

4.1.4 Roofwater

As part of the AGWR stormwater guidelines, roofwater was considered in scope. Data from a range of sources was reviewed and the data from a New South Wales Health monitoring program was considered to be the most useful and was used to support guidelines. Only bacteria were considered relevant. The concentrations were set at 0.06 bacteria per L of raw roofwater as a 95th percentile.

Table 4-1. Default 95%ile pathogens per L in the AGWR series (NRMMC *et al.*, 2006, 2008 and 2009).

Pathogen group	Sewage	Greywater*	Stormwater	Roofwater
Protozoa	2,000	20	1.8	N/A
Viruses	8,000	80	1	N/A
Bacteria	7,000	70	15	0.06

**The greywater default values given in the AGWR are examples and case-by-case assessment is recommended.*

5 More recent evidence

5.1 Raw data

The purpose of this Milestone 3 of Sub-project 4 was to critically review the default pathogen concentrations currently given in the AGWR that form that default start point for pathogen reduction validation for almost all recycled water schemes in Australia. The objective was to review and if, and only if, required, the objective was to update parameters such as:

- the choice of reference pathogens for which validation is required;
- the simple summary concentration statistics for use in deciding the start point for determining pathogen log reductions; and
- to provide probability density functions to enable more probabilistic pathogen concentrations to be used as inputs to more sophisticated methods of microbial risk assessment and process validation, such as the approach described in the core of Sub-project 4.

In order to characterise the both the concentration and variability in pathogen concentrations and to enable statistical comparison and pooling of data it was unfortunately necessary to trouble data custodians to share raw data rather than just summaries (e.g. averages). In addition, it was necessary to know what methods had been used and to have the opportunity to discuss these with laboratories. It was important that the correct inferences were made with respect to factors such as the specificity and recovery efficiency of the methods utilised. At the time of writing some follow-ups with laboratories are still in progress to help butter understand the data.

5.2 Data transformations

5.2.1 Less than values

For producing the simple summary statistics any "<" values were substituted with half the reported lower enumeration limits. This transformation had little or no effect on the most important descriptive summary statistics used in assessing health risks (medians, upper percentiles and maxima) but did influence minimum values. Therefore, where appropriate, minimum values were expressed as < the relevant enumeration limit.

5.2.2 Greater than values

Some datasets had numerous ">" values, in some cases constituting significant proportions of the data set. Such results would potentially significantly affect the most important descriptive summary statistics used in assessing health risks (medians, upper percentiles and maxima). Fortunately, alternative datasets were available so that whilst those datasets were valuable to an extent and were taken into consideration as part of the overall review, the presence of these heavily right-censored datasets did not significantly influence the final results as they were not ultimately utilised in the more in depth analysis. Where some isolated > values were retained within the dataset these were substituted with twice the upper limit of detection.

5.2.3 Recovery adjustments

Microbial count data reported alongside either parallel split sample positive controls, or internal standard positive controls, had their concentrations adjusted for that recovery. Where split sample controls were used the same day controls were used to adjust the sample values rather than a pooled average or frequency distribution based on multiple controls. Similarly, where internal standard recoveries were used the recovery adjustments to the reported sample value were made based on the internal standard from the same sample.

Note that in some cases no recovery data was available. In such cases it has been necessary to take the data at face value. Such data would underestimate pathogen concentrations but not by a known quantity. The summary tables indicate whether or not data been adjusted for recovery.

5.2.4 Confirmation

Where results were confirmed using differential interference contrast (DIC) microscopy or 4',6-diamidino-2-phenylindole (DAPI) staining the values reported here have been adjusted to only include so-called confirmed cysts and all assists.

5.3 Raw sewage

5.3.1 Cryptosporidium

The broad body of data showed that the choice of summary statistic for *Cryptosporidium* is quite appropriate. Even factoring in seasonal peaks (discussed below in Section 5.3.6) the AGWR default 95%ile value of 2,000 *Cryptosporidium* oocysts per L appears reasonably sound. However, there was approximately one order of magnitude difference between STPs managed by cities 1 and 2 as distinct from city 3 (Table 5-1 and Figure 5-1) and the reason for this difference is currently being investigated. Therefore, at this stage, these conclusions are interim. Taken at face value it seems unlikely that these differences reflect true differences in pathogen carriage rates between otherwise very similar cities, although that cannot be conclusively ruled out and is being considered. It is considered more likely that the differences reflect methodological variables and these are being followed up to establish whether or not one dataset is in fact more representative than another.

Table 5-1. Raw sewage *Cryptosporidium* concentrations per L reported across Australia. Data from three cities: two STPs in city #1, two locations at one STP in city #2 (a: screened and b: primary) and pooled from five STPs in city #3.

Parameter	AGWR default	1a	1b	2a	2b	3a-e
DAPI confirmed?		?	Y	Y	Y	Y
Internal standard recovery adjusted?		Y	Y	Y	Y	Y
Median		146	212	131	188	13
Arithmetic mean		455	1,591	212	489	23
95%ile	2,000	1,651	5,469	619	1,313	52
Maximum		6,875	42,667	909	3,934	242
Standard deviation		1,010	5,331	237	865	38
Samples		127	73	20	20	44

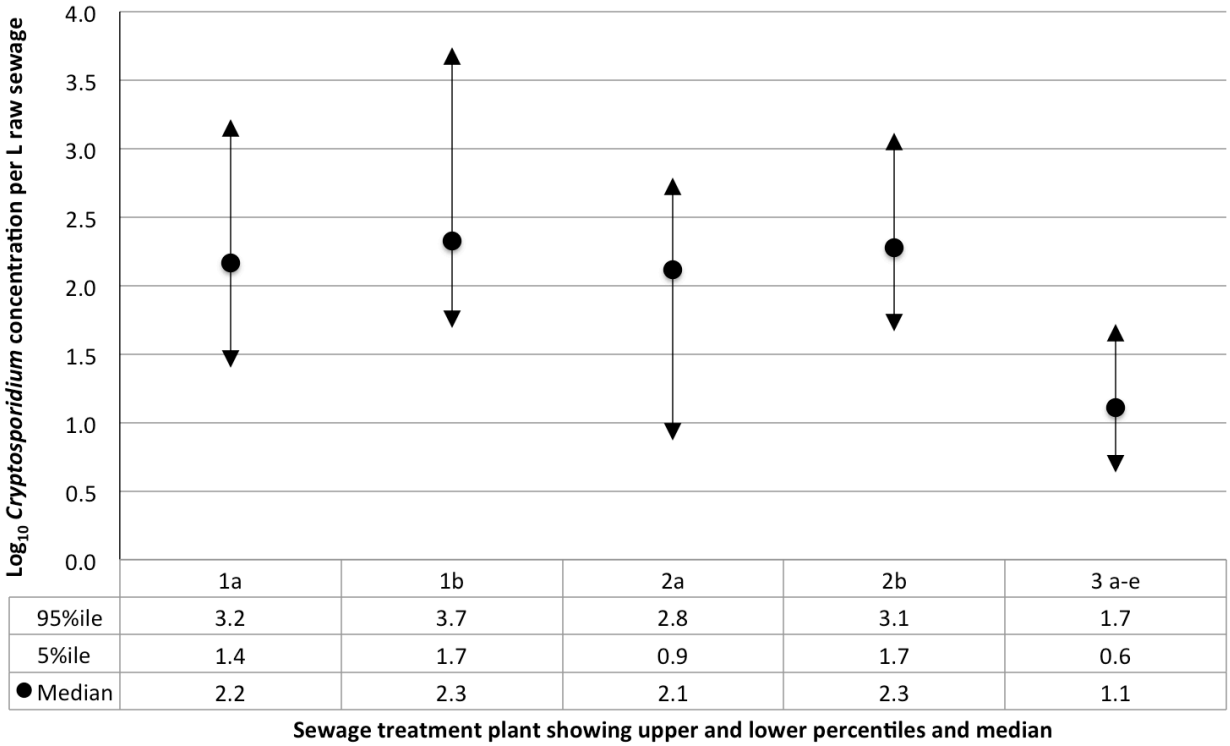

Figure 5-1. Log_{10}-transformed median, 95th and 5th percentile raw sewage *Cryptosporidium* concentrations per L reported across Australia. Data from three cities: two STPs in city #1, two locations at one STP in city #2 (a: screened and b: primary) and pooled from five STPs in city #3.

5.3.2 Giardia

There is no summary statistic given in the AGWR for *Giardia*. However, since suitable data was available it was assessed and reviewed. Taking the value of 2,000 *Cryptosporidium* oocysts per L as a default for *Giardia* provides a number below that applicable to STPs 1a, 1b and 2a (Table 5-2 and Figure 5-2) for *Giardia* and this is discussed further below (Section 5.3.5). Furthermore, the reason for the order of magnitude difference between various cities, and various STPs and raw sewage sampling points in of the same STP, are currently being investigated. Therefore, at this stage, these conclusions are interim. As noted for *Cryptosporidium*, it seems unlikely that differences reflect true differences in pathogen carriage rates between STPs in the same city or between otherwise very similar cities, although that cannot be conclusively ruled out and is being considered. Once again, it is considered more likely that the differences reflect methodological variables and these are being followed up to establish whether or not one dataset is in fact more representative than another.

Table 5-2. Raw sewage *Giardia* concentrations per L reported across Australia. Data from three cities: two STPs in city #1, two locations at one STP in city #2 (a: screened and b: primary) and pooled from five STPs in city #3.

Parameter	AGWR default	1a	1b	2a	2b	3a-e
DAPI 'confirmed'?		?	Y	Y	Y	Y
Internal standard recovery adjusted?		Y	Y	Y	Y	Y
Median		2,500	4,000	5,728	1,285	311
Arithmetic mean		3,442	8,033	16,764	1,446	1,023
95%ile	[2000 - *Cryptosporidium*]	8,025	32,985	55,583	3,207	4,029
Maximum		27,000	73,895	121,650	5,195	8,823
Standard deviation		3,557	13,453	27,588	1,203	1,737
Samples		150	73	20	20	44

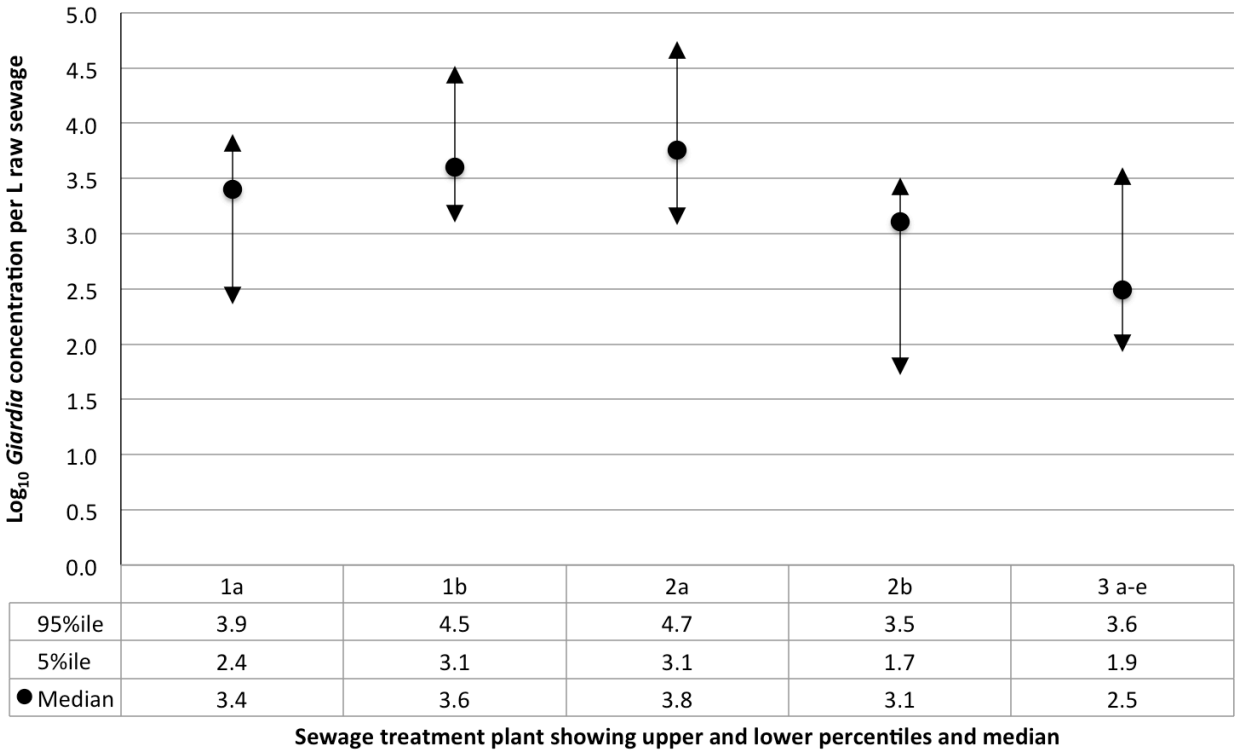

Figure 5-2. Log_{10}-transformed median, upper 95th and lower 5th percentile raw sewage *Giardia* concentrations per L reported across Australia. Data from three cities: two STPs in city #1, two locations at one STP in city #2 (a: screened and b: primary) and pooled from five STPs in city #3.

5.3.3 Adenovirus

The broad body of data showed that the choice of summary statistic for viruses is appropriate when compared to the MPNIU assay method, which was the method used to provide the evidence that underpins the current AGWR (Table 5-3 and Figure 5-3). The qPCR methods do not measure viability and are, therefore, not necessarily appropriate for health risk assessment in this context. Similarly, the MPNIU CCPCR method enumerates viral constituents that are not all likely to be representative of the concentration of viruses that would be infectious *in vivo* (White P, et al., in preparation). Therefore, the data to be utilised to represent viral pathogen concentrations in wastewater should be based on the MPNIU assays at this time. However, it was noted that good quality spikes were used for the analysis based on the MPNIU CCPCR method and these permitted recovery adjustment – something not undertaken for the MPNIU results shown nor for the data utilised to support the development of the current AGWR. Therefore, it is recommended that future studies should, as far as practicable, include suitable recovery controls, as are routinely used for protozoan pathogen assays.

Table 5-3. Raw sewage (log_{10} transformed) adenovirus concentrations per L reported across Australia. Data from three cities: two STPs in city #1, two locations at one STP in city #2 (a: screened and b: primary) and pooled from five STPs in city #3.

Parameter	AGWR default	1a	1b	1a	1b	2a	2b	3a-e
Assay type		qPCR		MPNIU		qPCR		MPNIU CCPCR
Spilt sample spike recovery adjusted?		N	N	N	N	Y	Y	Y
Median		4.5	8.0	2.9	3.4	7.1	7.3	5.8
Arithmetic mean		4.9	8.1	3.0	3.5	8.7	7.6	6.1
95%ile	3.9	5.4	8.5	3.4	4.0	9.1	8.0	6.7
Maximum		5.5	8.8	3.4	4.3	9.9	8.4	6.9
Standard deviation		4.9	8.1	2.9	3.5	9.3	7.7	6.2
Samples		21	74	19	73	20	20	43

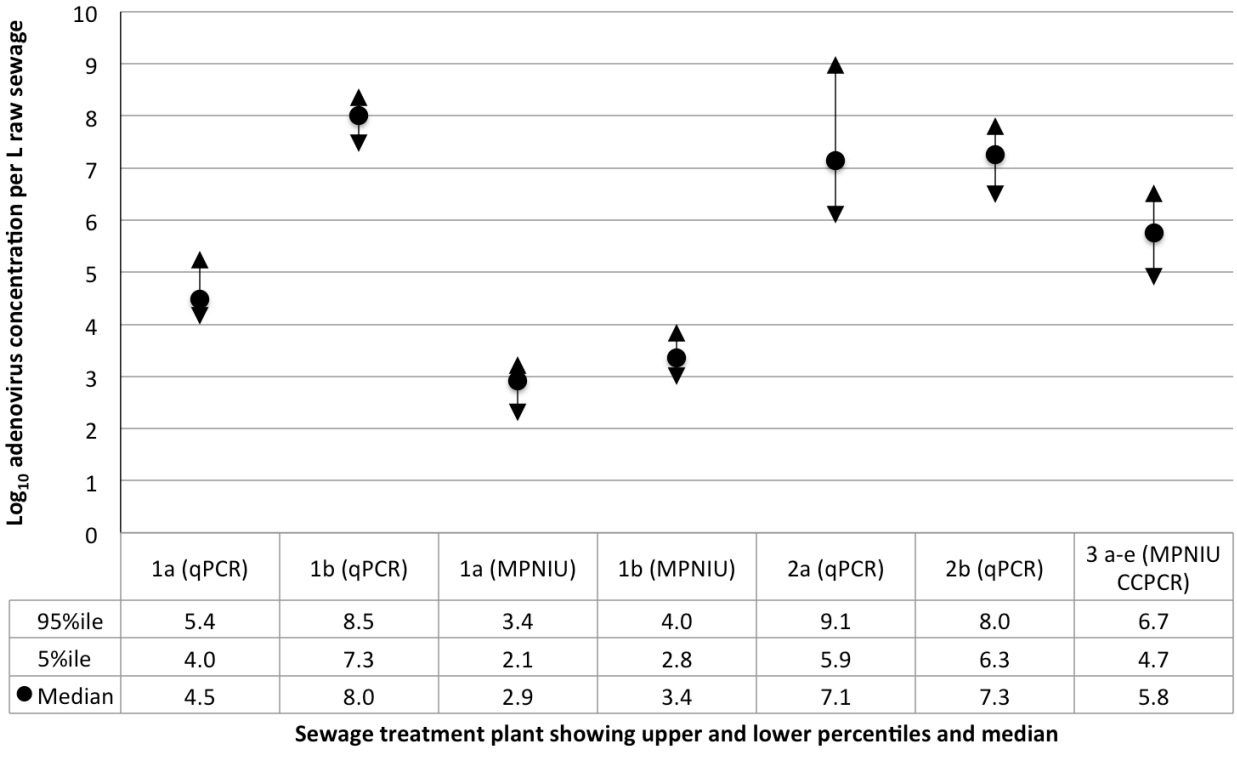

Figure 5-3. Log_{10}-transformed median, upper 95th and lower 5th percentile raw sewage virus concentrations per L reported across Australia. Data from three cities: two STPs in city #1, two locations at one STP in city #2 (a: screened and b: primary) and pooled from five STPs in city #3.

5.3.4 Salmonella

Only one reliable sample set was provided for pathogenic bacteria and although limited the data was derived from a study that covered five STPs, albeit in one city. The data implied that that the *Campylobacter* concentration given in the AGWR is possibly inadequate (Table 5-4 and Figure 5-4), at least to protect from *Salmonella*. There may be potential justification, following further investigation and peer review, to consider an increase in pathogen reduction requirements of 1 to 2 $\log_{10}$. In practice this would have little consequence for most recycled water schemes since it is almost invariably the viruses or protozoa that are overwhelmingly limiting and bacteria are typically amply taken care of by default. Therefore, at the time of writing, this finding is not being investigated further.

Table 5-4. Raw sewage ($\log_{10}$ transformed) *Salmonella* concentrations per L reported. Data pooled from five STPs in the same city.

Parameter	AGWR default	3a-e
Median		3.7
Arithmetic mean		4.7
95%ile	[3.8 for *Campylobacter*]	5.3
Maximum		6.0
Standard deviation		5.2
Samples		44

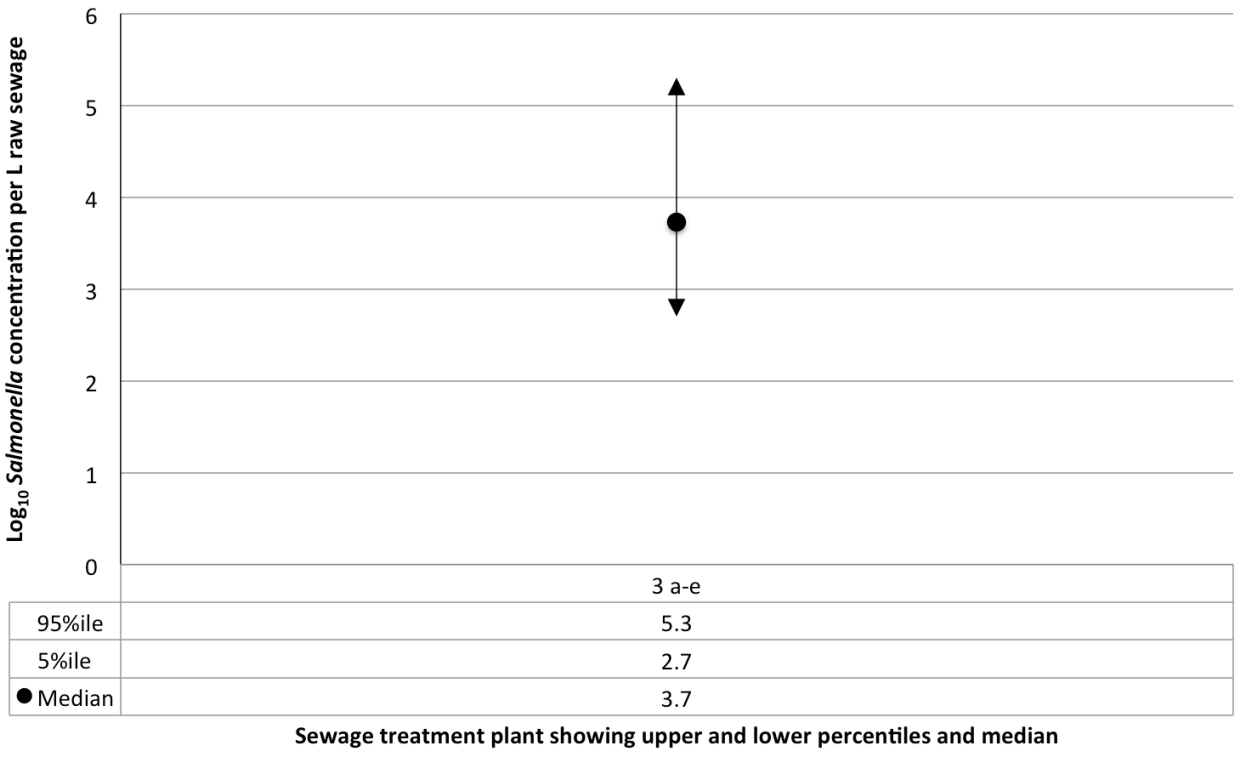

Figure 5-4. Log_{10}-transformed median, upper 95th and lower 5th percentile raw sewage *Salmonella* concentrations per L reported pooled from five STPs in one city.

5.3.5 Choice of reference pathogen for the AGWR

5.3.5.1 Protozoa

Where collected, the simple summary statistics and the magnitude parameters of the fitted frequency distributions in raw, screened or primary sewage were higher, by just over one order of magnitude, for *Giardia* than they were for *Cryptosporidium* concentrations. Whilst the latter is more environmentally persistent and more resilient to most means of treatment, caution is required in using *Cryptosporidium* to derive the recommended protozoan pathogen concentration to assume for the start point of validation studies. It is important not to overlook *Giardia* in situations where its reduced inherent stability does not drop its concentration below that of *Cryptosporidium*, e.g. in situations where there is a minimal level of treatment and exposure controls represent the principal means of protection against disease transmission. Therefore, whilst no change to the current AGWR position on the choice of reference pathogen for estimating protozoan pathogen concentration in raw sewage is necessarily implied, a strong caveat is required to cover certain scenarios and in such cases *Giardia* should be considered. These cases would include situations where minimal treatment was provided and exposure could occur quite soon after recycled water was provided.

5.3.5.2 Viruses

Where collected, the simple summary statistics and the order of magnitude of the parameters of the fitted frequency distributions were lower for enterovirus concentrations (data not shown in this report) than they were for the adenoviruses. Similar data for norovirus found once again that adenoviruses were much more numerous (data not shown in this report). Therefore, the adenovirus data was consistently the most elevated and, as was the case during the original development of the AGWR, adenovirus should be selected when estimating pathogen concentrations as the start point for validation studies. As a result no change to the current AGWR position on the choice of reference virus for estimating viral pathogen concentrations in raw sewage is implied by these results.

5.3.5.3 Bacteria

There was insufficient bacterial pathogen data collected over the past ten years to justify any change in the current AGWR position on the choice of reference bacterium for estimating bacterial pathogen concentration in raw sewage. The only reliable dataset provided for the purposes of this project was for *Salmonella* and not *Campylobacter*. The current reference pathogen, *Campylobacter*, has a higher propensity to cause infection than *Salmonella* (i.e. the probability of infection for a given dose of pathogen ingested is higher for *Campylobacter* than *Salmonella*). However, given the greater environmental persistence, and indeed environmental amplification propensity, of *Salmonella*, it is recommended that either both bacteria be considered, particularly in situations where food crop irrigation may occur, or that *Salmonella* be utilised as the reference pathogen in place of *Campylobacter*.

5.3.6 Spatial and temporal variations

5.3.6.1 Frequency distributions

The only simple summary statistics that differed when summarising log-transformed rather than untransformed data are the arithmetic average (mean) and the standard deviation. The other statistics remained the same if reverse-transformed. However, for Sub-project 4 it is necessary to understand not just the summary statistics but also the shape or pattern of pathogen concentrations.

In some cases pathogen concentrations vary consistently and can be fitted to a probability density function (such as the normal distribution, although more usually to the lognormal, Poisson or negative binomial distribution). However, other datasets are somewhat chaotic and are best characterised by a conventional baseline probability density function but allowing for overlying events to occur, time series or other forms. Therefore, it was necessary to characterise those distributions based on the data available from the generous data custodians that supported this third Milestone of Sub-project 4.

In this case the data were found to fit well to lognormal and negative binomial distributions but not to normal or Poisson distributions. As an example Figure 5-5 shows some typical pathogen frequency distributions to provide an indication of the approximate magnitude of variation associated with best-fitting probability density functions. The actual choice of frequency distribution to fit to any particular data set for any future studies depends on which part of the distribution is of most interest (e.g. upper, central or lower portions) and how much of the dataset is left- or right-censored (e.g. no-detect data).

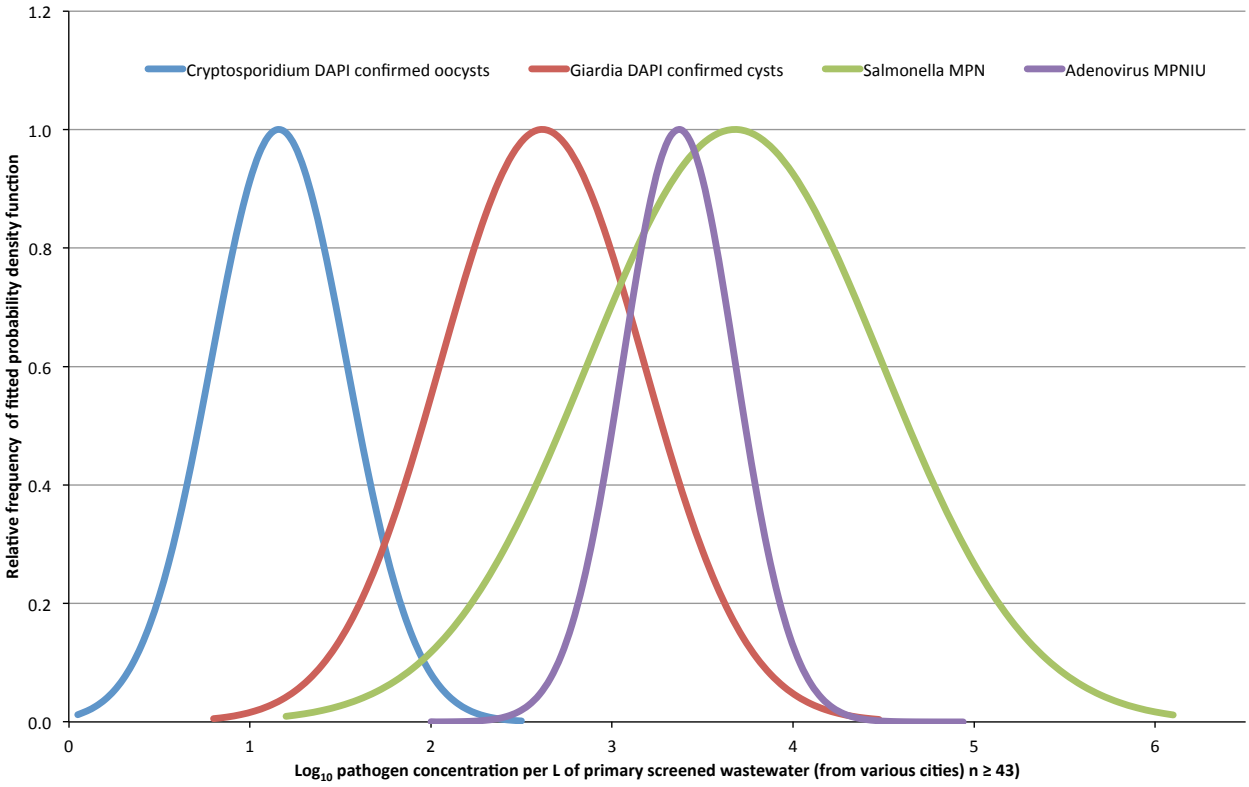

Figure 5-5. Best-fitting probability density functions fitted to log_{10}-transformed pathogen concentration data pooled from various STPs and cities.

5.3.6.2 Sampling point

The results revealed some inconsistent data for screened and primary settled sewage. In many cases primary settled sewage was the first point in the process train at which pathogens could be reliably monitored and analysts tended to ignore their own screened sewage results even if they had them. Furthermore, in some cases, where screened and primary settled sewage were both available, pathogen concentrations were higher in one or the other. For instance, *Giardia* and virus concentrations were higher in screened sewage prior to primary settling whereas *Cryptosporidium* concentrations were higher in the primary settled samples. In practice both have been considered interchangeably in most sampling programs and the differences are typically small. It is recommended that for future sampling studies the most suitable sampling point be selected taking into account practical sampling considerations.

5.3.6.3 Sewage treatment plant

There is no obvious reason why raw sewage at more than one large STP in the same city would differ. In many cases it is possible that differences reflect methodological variations rather than true differences. More sampling of more STPs over longer periods of time using carefully controlled and comparable methodologies are required to investigate this further.

It is by no means evident that pathogen concentrations would necessarily differ markedly between major Australian cities at similar times of year, although in some cases this is more plausible than variations between STPs within cities. Once again differences are likely to reflect methodological variations rather than true differences in many cases. More sampling of more cities over longer periods of time using carefully controlled and comparable methodologies are required to investigate this further.

5.3.6.4 Timeframe

What is clear is that there are some striking seasonal patterns and variations over time. Some pathogens are more commonly associated with winter or summer peaks, for instance, and examples were provided of datasets that reveal those differences. A large two-year dataset showed two summer peaks for *Cryptosporidium* – consistent with the widely publicised rise in cryptosporidiosis acquired during periods when swimming

increases. This effect is illustrated in a time series for *Cryptosporidium* (Figure 5-6) which is contrasted with a times series for *Giardia* (Figure 5-7).

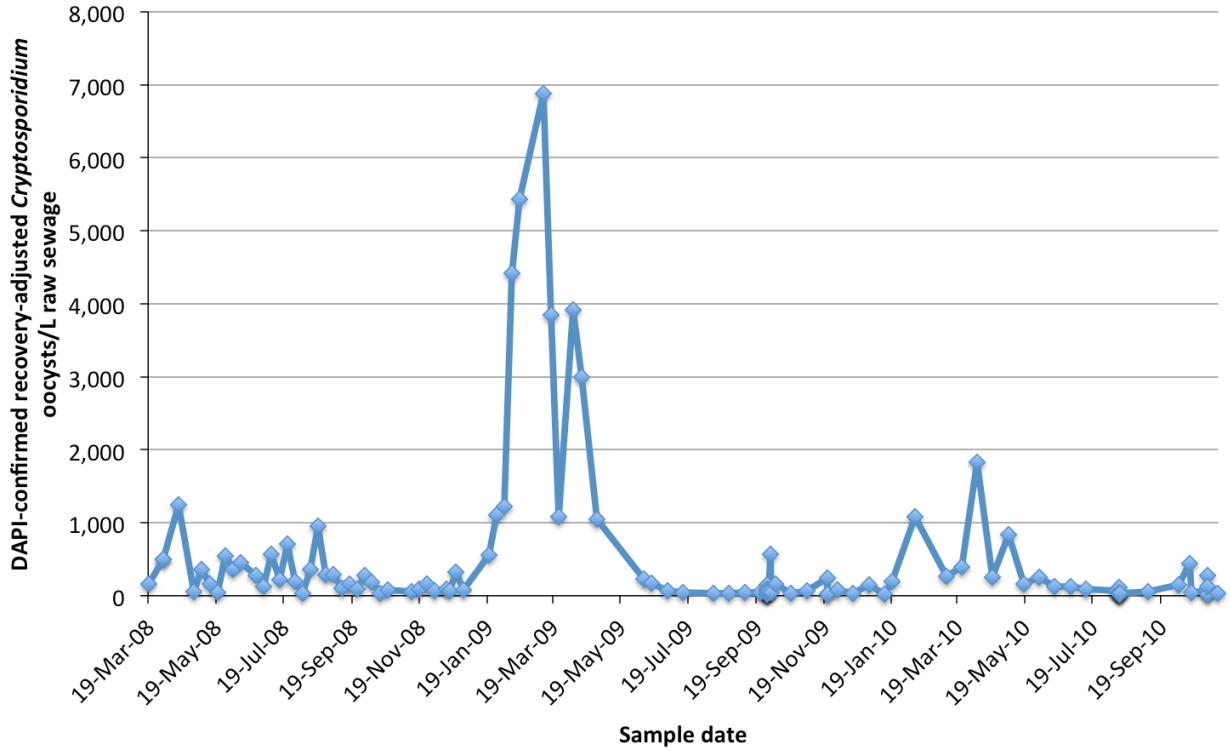

Figure 5-6. Example of *Cryptosporidium* monitoring showing concentrations rising during summer. The 2009 peak is more pronounced than the 2010 peak but both are clearly evident.

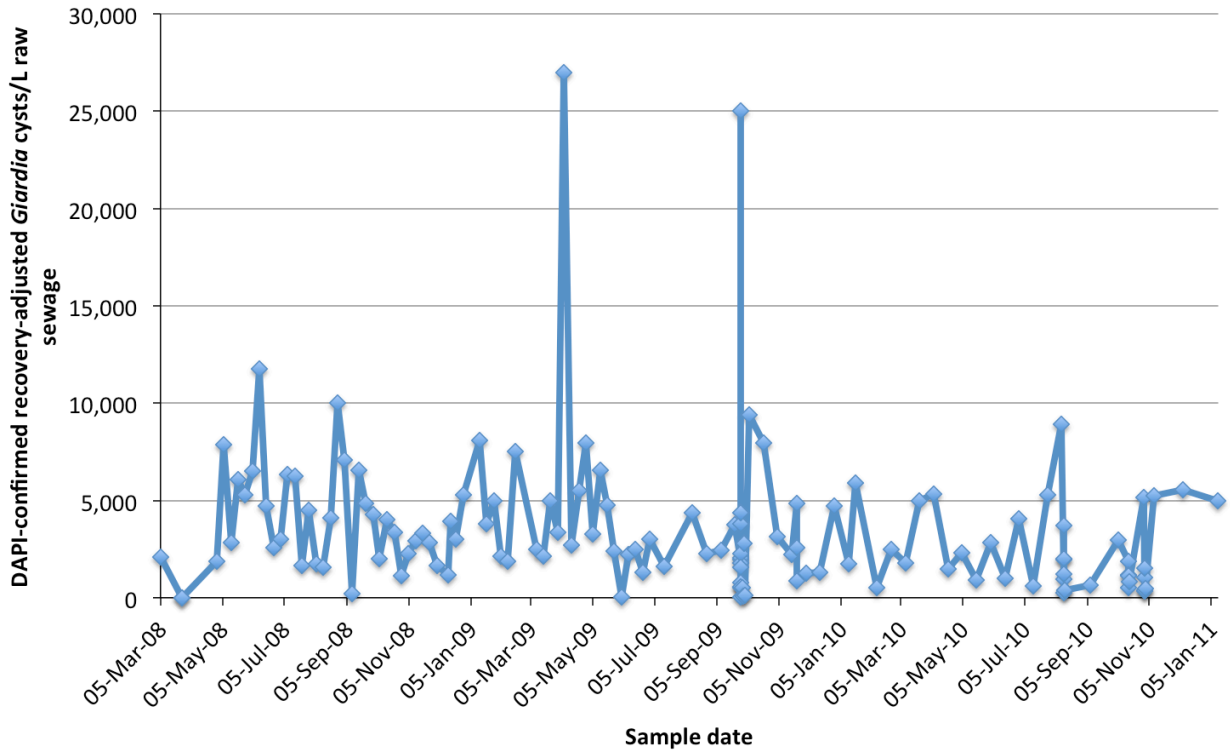

Figure 5-7. Example of *Giardia* monitoring showing no self-evident concentration rise during summer. There are two outlying high level samples but those are not evidence of a seasonal pattern.

5.4 Pathogen concentrations in Australian secondary treated effluent

Pathogen concentrations were markedly reduced between the screened and primary effluent raw sewage sampling points and the sampling points following treatment by an activated sludge process (ASP) and, in one case, ASP followed by a maturation pond. However, microbial reduction measured across the ASPs varied in several respects: i) between plants; ii) between statistics selected for the determination of microbial reduction (e.g. median, mean or 95%ile); and iii) between sampling points selected to represent raw sewage (e.g. screened or primary effluent). In the following summary the $\log_{10}$ reduction is taken to be the difference between the median statistics of the first sampling point in the process train (screened sewage) and the effluent from the ASP. In one case the treated water sampling point is downstream of a maturation lagoon as well as an ASP.

5.4.1 Cryptosporidium

Between screened sewage and ASP effluent *Cryptosporidium* oocyst concentrations were reduced by approximately 0.9 to 2.6 $\log_{10}$ (Table 5-5), consistent with the AGWR default values of 0.5 to 1.5 $\log_{10}$. The current convention of assuming 0.5 $\log_{10}$ reduction as a conservative default for uncharacterised ASPs appears to be credible as do the typical ranges of *Cryptosporidium* oocyst reduction by ASPs given in the AGWR.

Table 5-5. Secondary treated sewage *Cryptosporidium* concentrations per L reported across Australia. Data from two cities, two STPs in city (1) and one STP in city (2).

Parameter	AGWR default	1a	1b	2
DAPI confirmed?		?	Y	Y
Internal standard recovery adjusted?		Y	Y	Y
Median		18	0.6	3
Arithmetic mean		87	1.7	6
95%ile		392	6.2	25
Maximum		1,545	14.5	28
Standard deviation		192	2.5	8
Samples		199	75.0	20
Log_{10} reduction	0.5 to 1.5	0.9	2.6	1.7

5.4.2 Giardia

Between screened sewage and ASP effluent *Giardia* cyst concentrations were reduced by approximately 1.7 to 4.2 $\log_{10}$ (Table 5-6), consistent with the AGWR default values of 1.0 to 2.0 $\log_{10}$. The current convention of assuming 1.0 $\log_{10}$ reduction as a conservative default for uncharacterised ASPs appears to be credible as do the typical ranges of *Giardia* cyst reduction by ASPs given in the AGWR.

Table 5-6. Secondary treated sewage *Giardia* concentrations per L reported across Australia. Data from two cities, two STPs in city (1) and one STP in city (2).

Parameter	AGWR	1a	1b	2
DAPI 'confirmed'?		?	Y	Y
Internal standard recovery adjusted?		Y	Y	Y
Median		8	0.3	121
Arithmetic mean		109	2.0	157
95%ile		529	10.9	371
Maximum		3,600	19.7	800
Standard deviation		399	3.7	171
Samples		178	75.0	20
Log_{10} reduction	1.0 to 2.0	2.5	4.2	1.7

5.4.3 Adenovirus

Between screened sewage and ASP effluent virus concentrations were reduced by approximately 0.7 to > 8.3 log_{10} (Table 5-7), consistent with the AGWR default values of 0.5 to 2.1 log_{10}. The current convention of assuming 0.5 log_{10} reduction as a conservative default for uncharacterised ASPs appears to be credible as do the typical ranges of virus reduction by ASPs given in the AGWR.

Table 5-7. Secondary treated sewage (log_{10} transformed) adenovirus concentrations per L reported across Australia. Data from two cities, two STPs in city (1) and one STP in city (2).

Parameter	AGWR	1a	1b	1a	1b	2
Assay type		qPCR		MPNIU		qPCR
Spilt sample spike recovery adjusted?		N	N	N	N	Y
Median		3.8	< 1	0.9	< 1	4.5
Arithmetic mean		4.2	2.0	1.1	< 1	4.5
95%ile		4.8	< 1	1.6	< 1	5.2
Maximum		4.8	3.6	2.0	< 1	5.4
Standard deviation		4.3	N/A	1.3	N/A	0.6
Samples		21	75	52	75	20
Log_{10} reduction	0.5 to 2.1	0.7	> 8.3	2.1	> 3.5	2.6

5.4.4 Pathogen reduction across secondary treatment processes

The pathogen reductions given in the above paragraphs and tables are summarised graphically in Figure 5-8. The results demonstrate the value of characterising ASP and lagoon processes for pathogen reduction since it is clear that most ASPs perform better than the conservative defaults given in the AGWR. However, the margin of conservatism in the AGWR is not excessive. The very high reductions given for STP 1b are not typical as the plant includes both secondary ASP and a maturation lagoon before the sampling point.

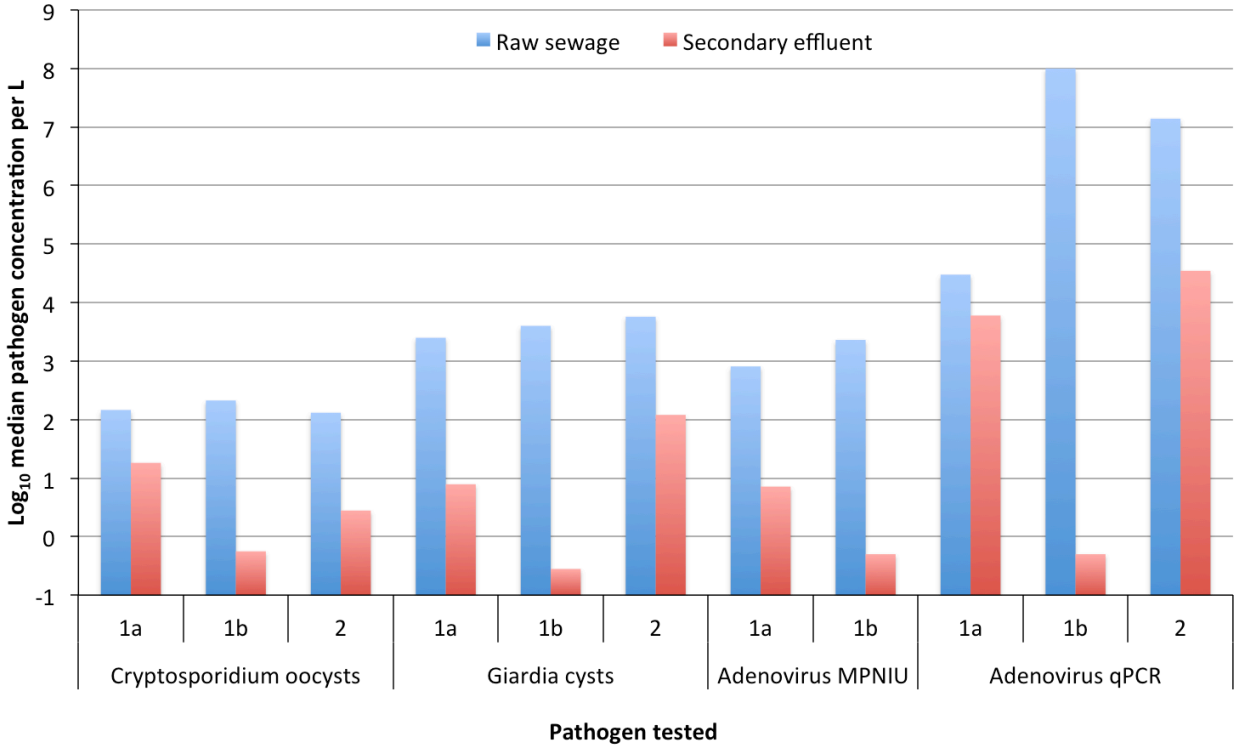

Figure 5-8. Log_{10}-transformed median pathogen concentrations per L comparing raw and secondary treated effluent. Data from two cities, two STPs in city (1) and one STP in city (2).

6 References

NRMMC-EPHC-AHMC (Natural Resource Management Ministerial Council, Environment Protection and Heritage Council, Australian Health Minister's Conference) (2006). *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks. Phase 1*. Australian Government.

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