AN APPROACH TO MANAGING WATER QUALITY IMPACTED BY TREATED SEWAGE

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ABSTRACT

As competing pressures from residential development and commercial shellfish-growing increase, it is necessary to maintain water quality as new knowledge about pathogens and their prevalence emerges. Viruses in sewage discharges are a particular hazard as, unlike bacteria, they may survive for weeks or months in the environment and can be infective at low doses. Viruses causing gastroenteritis are typically transmitted by the faecal-oral route, therefore may be present in sewage. Sewage discharges can lead to a public health risk from consumption of contaminated shellfish or ingestion of recreational water. Current approaches for controlling receiving water quality include an assessment of the impact on the receiving environment and/or managing the quality of the wastewater at the treatment plant. In New Zealand, there are no microbiological guidelines for wastewater quality, as the guidelines relate to the receiving environment. These guidelines warn that the relationship between indicator bacteria and viruses needs to be established for each point source discharge of treated sewage. This would require a risk assessment for every sewage discharge where there is a likelihood of human exposure. An alternative approach is to have a guideline which requires wastewater treatment plants to remove viruses before discharge. This paper summarises the derivation of a water quality criterion developed for the Mangere Wastewater Treatment Plant discharge in Auckland, New Zealand through application of the principles of risk management. It reviews the applicability of that virus removal criterion as a management tool in other locations. Given national and international water quality management strategies and recognising the difficulties of efficiently measuring viruses in water, an efficacious approach would be to prepare guidelines based on a tiered risk assessment approach.

KEYWORDS virus, microbial indicators, water quality, guidelines, wastewater

1 INTRODUCTION

Water quality is integral to New Zealanders' quality of life, our culture (e.g. food gathering/ mahinga kai), as well as giving economic benefits by providing competitive advantages and natural capital for major industries such as aquaculture and tourism. A function of our fresh- and marine-water resources is to safely assimilate treated sewage. To maintain good water quality as the competing pressures from residential development and commercial shellfish-growing increase, it is necessary to implement management practices consistent with new knowledge about pathogens, particularly viruses. Viruses in sewage discharges are a particular hazard as, unlike bacteria, they may survive for weeks or months in the environment and can be infective at low doses (Teunis et al., 2008).

National guidelines and international standards have been used to manage receiving water quality. However, use of bacterial indicators for shellfish-growing water quality inadequately protects shellfish from viral contamination in some cases overseas (Romalde et al. 2002*)* New Zealand's microbial guidelines for recreational areas (Ministry for the Environment, 2003) specifically exclude waters receiving sewage. In managing water resources for food gathering or recreation, two critical issues are:

- Point of control ,
  - at source in the wastewater treatment plant? Or
  - the receiving environment?
- Choice of indicator – bacteria or virus?

This paper summarises the derivation of a water quality criterion developed for the Mangere Wastewater Treatment Plant (WWTP) in Auckland, New Zealand (DRG, 2002), and review the applicability of a virus removal criterion as a management tool along with international management approaches. Application of the

principles of risk management used in New Zealand and overseas approaches to managing water quality are discussed. We also discuss the implications for measuring water quality from a literature review on indicators and propose a tiered approach to risk management.

2 WASTEWATER TREATMENT PLANT DISCHARGE QUALITY

Many countries prescribe the quality of the wastewater discharged from the WWTP, e.g. the UK requires disinfection for discharges to controlled areas where water quality standards apply for bathing or shellfish waters. However, New Zealand regulations (Resource Management Act, 1991) and guidelines (Ministry for the Environment, 2003) relate only to the receiving environment. From a practical perspective guidelines relating to the quality of the discharge are simpler to implement, compared to undertaking a risk assessment for each discharge to ensure that public health is protected. In some areas a virus criterion has been applied to WWTP in New Zealand, derived from a quantitative microbial risk assessment (QMRA) for discharge of wastewater from the Mangere WWTP to the marine environment. This section assesses the applicability of a criterion developed through a QMRA to other areas, by reviewing the components of the model and the assumptions.

2.1 DERIVATION OF A VIRUS REDUCTION CRITERION FROM A QUANTITATIVE MICROBIAL RISK ASSESSMENT

During the upgrade of the Mangere WWTP, there was considerable debate about the potential public health effects on nearby bathing and shellfish gathering activities. A QMRA was undertaken to determine a virus criterion for the discharge. The model identified 4-log enteric virus was required. This was determined as being achievable by the developer and adequate by the regulator. The wastewater treatment process at Mangere was based on the reduction of culturable human enteroviruses to below the limit of detection which was 1 enterovirus tissue culture infectious dose ($TCID_{50}$)/100L (Jacangelo et al., 2003) during normal conditions of influent virus concentration.

A human health risk assessment for the environment of the Mangere WWTP discharge (DRG, 2002) was undertaken for the two relevant exposure routes: aquatic recreation and shellfish consumption, using QMRA. Enteric viruses were determined to constitute the major infection risk to humans and so enterovirus and adenovirus were selected as the critical pathogens in the risk assessment. When running the stochastic model the acceptable level of risk of infection was set at 1 / 10,000 (as the 95th percentile*)*. This risk of infection is more conservative than used in other risk assessments for setting criteria e.g. 19 / 1,000 swims in sea water, 8 / 1,000 for swims in freshwater, or an annual risk not exceeding 1/10,000 for 95% of the time in the US drinking-water guidelines (DRG, 2002). The analysis showed that consumption of shellfish from the nearby reef was a higher risk than ingestion from bathing. The virus reduction criterion was therefore based on a model for shellfish consumption with the following components and assumptions:

1. Concentration of culturable enteroviruses and adenoviruses in Mangere raw sewage - no seasonal variation;
2. Wastewater flows (influent and various individual treatment processes);
3. Virus removal/inactivation by the treatment processes;
4. Virus concentrations in WWTP effluent as measured in the pilot plant study (Simpson 2003);
5. Virus survival in the harbour conservatively assumed to be 100% as travelling time to reef is just six hours;
6. Plume dispersion/dilution at shellfish-gathering site of 1:100 as modelled (1:100-300 modelled for shellfish-gathering site);
7. Bioaccumulation in filter-feeding shellfish 150-fold (maximum value found by Burkhardt and Calci 2000) and also assumed 24-hour immersion while the plume contacts the reef for only 2.5 hours on the incoming tide;
8. Estimation of shellfish serving size 60 or 200 g for low- and high- exposure scenarios, respectively (Rose and Sobsey, 1993);
9. Virus infectivity models the infectious dose being an estimate of the probability of someone who consumes contaminated shellfish becoming infected. Not everyone who is infected will become ill. Enterovirus was based on echovirus type 12: β-Poisson; $\alpha = 1.3$, $\beta = 75$ (Rose and Gerba, 1991) and infectious dose model for adenovirus: exponential; $r = 0.4172$ (Crabtree et al., 1997*)*.

2.2 APPLICABILITY OF 4-LOG VIRUS REMOVAL CRITERION TO OTHER LOCATIONS

Some of the QMRA components are internationally applicable. Bioaccumulation factors, shellfish meal size, virus infectivity data, even virus concentrations in wastewater discharges can all be taken from the literature, so is it appropriate to apply the criterion to other locations in New Zealand or internationally?

Several of the parameters used in the QMRA model (virus concentrations in the wastewater, virus removal, flows at Mangere WWTP, final virus concentrations and dilution) apply specifically to Mangere WWTP. One should therefore be cautious in applying the results of this QMRA to all WWTPs as the receiving environments are different. Each receiving environment will unique in terms of:

- dilution of the effluent plume, (local hydrology – currents, other freshwater inputs, tidal effects, period of time shellfish are immersed in water, if tidal);
- virus survival in the receiving environment (travel time, water temperature, salinity and turbidity);
- exposure to infection – how people would become infected (location of the recreational and shellfish growing areas in relation to the point of discharge from the WWTP and the type of shellfish.

2.2.1 VIRUS CONCENTRATIONS IN WASTEWATER TREATMENT PLANT INFLUENT AND EFFLUENT

A key factor to be considered in any QMRA model is that the concentrations of enteric viruses in sewage result from the level of viral excretion present in the local community. This will vary seasonally with different infection rates and the occurrence and extent of outbreaks. The requirement for a 4-log removal was based on the measured concentrations of viruses at Mangere WWTP at the time of the study.

A review of studies on enteric virus concentrations into and out of WWTPs was undertaken to illustrate typical ranges for wastewater influent and effluent and is summarised in Table 1. Only studies using culture methods are given as they provide data on infectivity and concentration. The most recent studies use polymerase chain reaction (PCR)-based methods to determine the presence or absence of a virus, consequently the concentrations of infective enteric viruses in wastewater are unknown in these studies. Direct comparison of the results of one study with another is not possible, unless the same method was used. There is no standard methodology for concentrating and determining the presence of enteric viruses by culture. The studies presented in Table 1 used a wide variety of methods to determine virus concentrations for different steps and consequently concentrations can be reported in different units. These differences can be significant, e.g. Hurst et al. (1988) reported concentrations of adenovirus five-times greater when using the 293 cell line compared with the HEp-2 cell line.

Table 1 shows a wide range of virus concentrations in the raw and treated sewage. Table 2 provides details of the WWTP. Influent and effluent virus data from New Zealand are reasonably comparable as the same cell line and generally the same method of concentration is used, except for Simpson et al. (2003*)* Enterovirus concentrations in New Zealand are low ranging from 1.7–3,900 pfu/L in the influent, and 0–180 in the effluent. In a recent study (Hewitt and Greening, 2005) adenovirus, concentrations in the influent ranged from 2–10,000 MPN IU/L, with 2–600 pfu/L in the effluent (Table 1*)* The maximum concentration for adenovirus and enterovirus measured at Mangere WWTP (Simpson et al., 2003) are around 1000 times more than recorded by any other study. Even over the longer surrogate study, maximum concentrations were elevated. The mean effluent concentrations in the studies in Table 1 are between 1–100 pfu/L for enterovirus. Adenovirus concentrations, as with enterovirus, have a wide range of concentrations.

Table 1 Summary of reported influent and effluent enterovirus and adenovirus data

Author	No.	Enterovirus pfu/L		Adenovirus pfu/L	
		Influent Mean	Effluent Mean	Influent Mean	Effluent Mean
Irving and Smith, 1981	26	1,400 (150-6,350) IU/L	100 (0-900) IU/L	1,950 (0-6850) IU/L	250 (0-600) IU/L
Irving and Smith, 1981 Chlorinated	7	NT	100 (0-250) IU/L	NT	300 (0-1,150) IU/L
Krikelis et al,. 1985		90-900 cu/L		70-3200 cu/L	NT
Dahling et al., 1989	15	Median 1,000 (100-242,500)	Median 600 (1-24,000)	NT	NT
Lewis et al,. 1986	12	2.28 (0-2.33)	1.41 (0-2.03)	NT	NT
	10/8	2.08 (0-2.52)	2.16 (0.3-2.45)	NT	NT
	6	1.7 (0.85-2.03)	2.51 (1.96-2.86)	NT	NT
	12	2.68 (0-3.33)	2.58 (1.18-3.37)	NT	NT
Hurst et al., 1988		1,625 $TCID_{50}$/L		146,040 $TCID_{50}$/L	NT
Asano et al., 1992	145	NT	Median 31.6 (<0.26-264.2) vu/L	NT	NT
	105	NT	Median <2 (<2->250) vu/L	NT	NT
	53	NT	Median 0.53 (1-734) vu/L	NT	NT
	60	NT	Median <0.44 (<0.44-136.59) vu/L	NT	NT
	47	NT	Median 2 (<0.0026-0.23) vu/L	NT	NT
Nasser et al., 1994	13	1-2	1-2 Max 18	NT	NT
Rose et al., 1996 biological treatment	60	GM 4.2 (0.54-45)	GM 0.053 (0133-1.1)	NT	NT
Rose et al., 1996 filtered	60	NT	GM 0.015 (<0.007-0.095)	NT	NT
Rose et al., 1996 chlorinated	60	NT	GM 0.0009(<0.004-0.006)	NT	
Moore et al., 1988[1]	3,3,2	NT	GM 50 spring 63 summer, 45 fall-winter	NT	
Greening et al., 2000	4	440 winter, 860 spring, 1,300 summer, 460 autumn	-, 2, 3.5, 0.3	10, <10, <10, <10	-, NT, NT, 0
Simpson et al., 2003 Surrogate study		50 percentile 2,000 (400-10,000) $TCID_{50}$/L	NT	NT	1,300 (1,100-11,000)$TDIC_{50}$/L
Simpson et al., 2003 Scoping study		1,000,000 (11,000-40,000,000) $TCID_{50}$/L	NT	NT	NT
Tree et al., 2003		400-700	NT	NT	NT
Leonard et al., 2003	9	2,000 (1,500-3,900)	Median 4 (1-32)	NT	NT
Hewitt & Greening, 2005 Small towns	3	(2–8)	(2-8)	(2-3,000) MPN IU/L	(2-100)MPN IU/L
Hewitt & Greening, 2005 Cities	3	(10-1,000)	(3-180)	(10-10,000)MPN/L	(2-600)MPN/L

Author	No.	Enterovirus pfu/L		Adenovirus pfu/L	
		Influent Mean	Effluent Mean	Influent Mean	Effluent Mean
Sedmark et al,.2005	112	0-12,820	0.5-26	NT	NT

Pfu=plaque forming units, VU=viral units, IU=infectious units, cu=cytopathic units, most probably number=MPN, GM=geometric mean, NT=not tested

* Calculated from reported value multiplied by 600 for concentration

Table 2 Details of study locations, period, and WWTP

Author	Wastewater Treatment Plant	Country	Period	Population/Flow
Irving & Smith, 1981	Activated sludge, with and without chlorination	Australia	1 year, every 2 weeks	>1,000,000
Krikelis et al., 1985	Various	Athens	15 months	>1,000,000
Dahling et al., 1989	10 trickling filters 5 activated sludge: 2 no chlorination	Puerto Rico	Once	950-26,000m^3/d
Hurst et al., 1988	No details	USA	mid Nov- mid Dec	
Lewis et al., 1986	WSP	NZ		600
	Biofilter	NZ		3000
	Sedimentation and biofilter	NZ		9000
	Sedimentation and chlorination	NZ		50000
Moore et al., 1988	Trickling filter , anaerobic digester supernatant	USA	3 year	30,000-49,000m^3/d
Asano et al., 1992	1 trickling filter, 2-5 activated sludge	USA	1-6 years	
Nasser et al., 1994	WSP	Israel	July - Oct weekly/2 weeks	900
Leonard et al., 2003	Trickling filter, WSP	NZ	2 months	300,000
Rose et al., 1996	Activated sludge, sand filtration, polymer, chlorination	USA	60 samples over 1 year	60,000m^3/d
Simpson et al., 2003	NA	NZ	Surrogate study weekly Oct-June Scoping study 3/week May-June	1,000,000
Tree et al., 2003	NA	UK		
Greening et al., 2000	Activated sludge	NZ		25,000
Sedmark et al., 2005	Activated sludge, phosphorus removal disinfection	USA	Monthly Aug 1994-July 2003	>1,000,000
Hewitt & Greening, 2005	Moving bed biofilm, trickling filter/activated sludge, activated sludge	NZ	3 times in summer	70,000-1,000,000
Hewitt & Greening, 2005	activated sludge, WSP, WSP with aeration	NZ	3 times in summer	1,000-10,000

Assuming that most methods give concentrations within one order of magnitude, the concentrations range from 1–40,000,000 pfu/L, the maximum value being in Auckland (Simpson et al., 2003). The extremely high values in Auckland and Puerto Rico (Dahling et al., 1989, Table 1) were believed to be related to the health of the communities at the time. This data illustrate that the maximum viral concentrations at Mangere were high compared to national and international studies.

Although the studies in Table 1 are only a snapshot, they illustrate that a blanket requirement for a 4-log reduction in virus concentration would appear excessive, except in the larger metropolitan areas where concentrations in the discharge and sensitivity of the receiving environment require such a reduction*)* Even a 3-log removal may be more than is required, when the receiving environment is taken into account. Except for one sample, enterovirus and adenovirus concentrations in studies reported by Lewis et al. (1986), Greening et al. (2000) and Hewitt & Greening (2005) were 0-600 pfu/L. This review shows that most virus removal in a WWTP is around 90-99% (1-2 logs), but higher removals can be achieved with well operated filtration and disinfection plants.

A generic requirement for a 4-log virus removal is not supported by the literature on culturable enteric virus (reovirus, adenovirus or enterovirus) concentrations in raw sewage. In conclusion, this criterion for WWTP quality should not be applied to other areas without determining the local environmental conditions, and measuring concentrations of culturable enteric viruses in the wastewater. Relying on literature values for enteric virus concentrations may be misleading.

3 CRITERIA FOR THE RECEIVING ENVIRONMENT

Not all receiving environments warrant the same level of protection from a human health standpoint. For example, discharges into waters used for growing commercial shell fish pose a much greater health risk than discharges into recreational areas, or high energy coasts with rapid mixing and unattractive beaches where few people are likely to swim. Not all WWTP therefore need to have the same quality of wastewater outputs. New Zealand's environmental legislation is site-specific. This allows the quality of the WWTP discharge (and consequently the type of WWTP) to be tailored to the physical conditions of the receiving environment, the use of that environment, cultural requirements and the cumulative effects on the environment from other discharges.

3.1 ENTERIC VIRUS SURVIVAL IN RECEIVING WATERS

Viruses are likely to be the most significant pathogens in terms of health risk discharged from the WWTP to water. Viruses may survive for long periods and travel long distances. In the natural environment, viruses are negatively charged and adsorb to different matrices (Gerba, 1984). Often they bind tightly to sand, clay and sediment particles that settle on the bottom leading to their accumulation in river, lake and marine sediments. Virus survival in water depends on a range of physical, chemical and biological factors, including water temperature, sunlight, salinity, pH, presence of particulate matter and natural microbial activity, of which sunlight and temperature (Moce-Llivina et al., 2005, Johnson et al,. 1997, Craig et al., 2002) are the most important factors. Microorganisms generally survive longer in dark, cool conditions where the ambient levels of microorganisms are low.

There is much literature about the survival of various microorganisms in water and wastewater. A summary is given in Table 3. The survival parameter generally used is a T_{90} value (the time taken to reach a 90% reduction – a 1-log reduction*)*. However, reproducible results are seldom obtained unless the survival experiments are conducted in a laboratory where the factors that impact on survival can be controlled. So while laboratory-based studies provide insight into the relative effects on survival of various environmental factors (e.g. temperature, salinity, pollution load), the T_{90} values often differ greatly from those obtained from field studies – compare the longer T_{90} values from filtered-sterilised seawater (Cranze et al., 1998) with Wait & Sobsey (2001) who used natural seawater (Table 3). The estimation of microbial survival may be unique to that particular set of experimental conditions and these are rarely repeatable in the field. Therefore, one must be cautious when comparing the survival rates of different microorganisms between different field experiments. Nevertheless, reliable comparisons can be made where several microorganisms are measured in the same survival trial and broad comparisons can be made between some trials, although absolute survival rates or T_{90} values should not be relied on too much.

Table 3 Examples of virus survival studies in seawater (SW)

Ref	Organism	T_{90}	T_{99}	time	Matrix type	Temp °C	Light/ dark	pH	Other conditions
Moce-Llivina et al., 2005	Somatic coliphage	53	114	h	SW + 1/50 raw sewage	24-25	Diurnal	7.7-8.1	Dialysis tube (water; 36-37‰ salinity; 0.8-1.8 NTU turbidity; 20-25 cm depth; summer
	F-RNA bacteriophage	14	33	h					
	Bacteroides thetaiotaomicron bacteriophage	95	191	h					
	Echovirus 6	36	74	h					
	Coxsackievirus B5	37	84	h					
Wetz et al., 2004	Poliovirus Lsc1	2.2	4.4	d	SW	22			
		2	4	d		30			
Crance et al., 1998	HAV CF53		stable	d	Synthetic SW, salinity=24g/L; filter-sterilised	4	Dark		Lab
			24	d		19			
			11	d		25			
Gantzer et al., 1998	Poliovirus 1	671		d	SW; filter-sterilised	4	Dark		Lab
		76		d		18			
		25		d		25			
		30		d					
		23		d					
		26		d					
		28		d					
		26		d					
		31		d					
		36		d					
Johnson et al., 1997	Poliovirus 1	10.3	20.6	h	Black Point Beach	22-26	Sunlight	8.0	Beaker glass/polyprop roof
		26	52	h			Dark	8.0	Beaker glass/polyprop lab
		10.8	25.7	h	Ala Wai Canal		Sunlight	8.2	Beaker glass/polyprop roof
		23	46	h			Dark	8.2	Beaker glass/polyprop lab
Wait & Sobsey, 2001	Poliovirus 1	10		d	SW 1.2km offshore, winter	6	Dark	7.9-8.3	Lab, glass flasks, shaken
		7.5		d	SW 1.2km offshore, spring				
		1.8		d	SW 1.2km offshore, summer				
		10.5		d	SW 1.2km offshore, autumn				

Ref	Organism	T_{90}	T_{99}	time	Matrix type	Temp °C	Light/ dark	pH	Other conditions
Wait & Sobsey, 2001	Poliovirus	5		d	SW 1.2km offshore, winter	12	Dark	7.9-8.3	Lab, glass flasks, shaken
		6.4		d	SW 1.2km offshore, spring				
		1.9		d	SW 1.2km offshore, summer	12			
		0.8		d	SW 1.2km offshore, autumn				
		2		d	SW 1.2km offshore, winter	20			
		3.1		d	SW 1.2km offshore, spring				
		1		d	SW 1.2km offshore, summer				
		1		d	SW 1.2km offshore, autumn				
		1.5		d	SW 1.2km offshore, winter	28			
		2.1		d	SW 1.2km offshore, spring				
		1		d	SW 1.2km offshore, summer				
		1.8		d	SW 1.2km offshore, autumn				
		7.1		d	SW 2km offshore, winter	4-7.5			Diffusion chamber depth 3m
		6.6		d	SW 2km offshore, winter				Diffusion chamber depth 10m
		2.6		d	SW 2km offshore, summer	22-24			Diffusion chamber depth 3m
		1.5		d	SW 2km offshore, summer				Diffusion chamber depth 10m
		1.7		d	SW 2km offshore, autumn	18-20			Diffusion chamber depth 3m
		0.8		d	SW 2km offshore, autumn				Diffusion chamber depth 10m
Nasser et al., 2003	Coxsackievirus A9	500		d	SW clean site conductivity= 56.6 ms	15		8.4	
		6.5		d		30			
		14		d	Brackish conductivity=13.9 ms	15		8.5	
		5.7		d		30			
Craig et al., 2002	Coliphage	5.3		d	SW Henley Beach	10			Microcosm in water bath
		2.5		d		20			
		1.7		d		30			
		6.3		d	SW Onkaparinga	10			
		2.9		d		20			
		2.0		d		30			
		8.3		d	SW Port Adelaide	10			
		5.0		d		20			
		1.8		d		30			

3.2 SETTING VIRUS CONCENTRATIONS IN RECEIVING WATER

No international regulatory virus standards for bathing water or shellfish-growing waters currently exist, although studies have been undertaken to determine the feasibility of using viral indicators. In Europe, a study is underway to determine the feasibility of using molecular methods for norovirus and adenovirus to manage bathing waters (Virobathe, 2009). Unpublished PCR results indicate that adenovirus was detected at least once in 22 of 24 sites (92%), while norovirus was detected in 17 of the 24 sites (71%) (Wyn-Jones pers. comm.).

In shellfish growing areas of the USA the Food and Drug Administration (USFDA) derived a value for water quality based on the accumulation of enteric viruses in the shellfish and consumption of a shellfish meal, for training purposes. The risk of enteric virus (enteric and enteroviruses used interchangeably in the training presentation) infection from contaminated shellfish using an infectious dose for enterovirus of 10 virus particles (a hypothetical example not a measured concentration), meal size of 20 oysters and a safety factor of 10. The concentration of viruses per oyster was calculated as 0.05 enteric virus particles (Goblick, pers. comm.). The critical value for shellfish-growing waters (< 0.04 enteric viruses / L) was then derived from a calculation using the assumptions in Kohn et al., 1993 that each oyster contains 25 mL water and there is a 50-fold minimum accumulation factor.

Such a standard is impractical because the measurement of total enteric viral load in waters is not readily achievable due to technical difficulties, particularly the availability of assays to recover and measure each virus type. Nevertheless, this approach is a fair starting point but it needs to be supported by new data and better analytical methods. It is particularly difficult to predict infection risk when the most important pathogen, norovirus, had, until recently no accepted infectious dose equation, no method of assessing infectivity, and no reliable indicator. However, recently Teunis et al. (2008) estimated that the median infectious dose for norovirus is about one virus. This is lower than used in the derivation of the water quality criterion which means that the derivation underestimates the risk of infection. The deterministic approach also oversimplifies the situation by assuming that all people are affected equally, which is not the case. Using Kohn et al. (1993) average values under-estimates the risk because most exposure variables have approximately log-normal distributions (i.e. are very right-skewed*)*

3.3 RECEIVING WATER STANDARDS AND GUIDELINES

In the absence of virus standards for water quality, current standards and guidelines are based on bacteriological criteria. Monitoring may be combined with other management practices such as sanitary surveys in New Zealand and the European Union (EU) or calculation of acceptable dilution of treated sewage by the receiving water for shellfish growing (USA*)*. In New Zealand, recreational and non-commercial shellfish growing waters are covered by guidelines (Ministry for the Environment, 2003), which state that even if microbial indicators are low, a sanitary survey may indicate high risk if sewage is discharged in the vicinity.

A comparison of the EU and New Zealand (NZ) guidelines show that acceptable concentrations vary depending on the indicator and local epidemiological studies (Table 3*)*. In particular, *Escherichia coli (E. coli)* concentrations in the New Zealand guidelines are much lower because the results of sanitary surveys are also included in the grading assessment. Conversely, the maximum enterococci guideline value is higher. This may be owing to the difficulties inherent in undertaking epidemiological studies. However, the New Zealand guidelines do not apply in waters into which sewage is discharged, so a risk assessment is required for every sewage discharge.

Table 3 Comparison of EU and NZ guidelines for recreational water quality.

	Indicator			
EU/New Zealand category		Excellent/A	Good/B	Sufficient/C
EU Freshwater	*E. coli*	500	1000	900*
New Zealand Freshwater	*E. coli*	≤130	131-260	261-550
EU Marine	*E. coli*	250	500	>500
EU Marine	Enterococci	100	200	185*
New Zealand Marine	Enterococci	<40	41-200	201-500
EU Freshwater	Enterococci	200	400	330

*90 percentile, all others are 95 percentile

3.4 DILUTION

WWTPs remove viruses – the reduction depending on the treatment used (Table 1). Discharge into the receiving environment will dilute them, reducing concentrations even further. The USFDA shellfish classification system is based on the quality of effluent being discharged from nearby WWTPs and the extent of effluent dilution in the receiving water. It links this to the travel time of the discharge to determine the shellfish-growing area boundaries. The boundary of the dilution zone defines the different zones within the shellfish-growing areas: approved; conditionally approved (allows for WWTP discharge quality failures); restricted. The primary compliance measure used in setting boundaries is 14 faecal coliforms / 100 mL in shellfish-growing waters. For conditionally approved areas, effluent dilution must be at least 1:1000 and there must be sufficient warning of WWTP failure to enable the shellfish harvesting to be suspended before the effluent reaches the boundary of the prohibited area[2]. For restricted areas, sufficient dilution of the effluent must occur during WWTP failure conditions for the faecal coliform median or geometric mean MPN of the water samples not to exceed 88/100 mL. The 1:1000 dilution which is required in the receiving environment, effectively provides a 3-log reduction in virus concentrations.

4 INDICATORS OF VIRAL PRESENCE IN WATER

As it is not possible to monitor for all enteric viruses possibly present in sewage or receiving waters, international practice has defaulted to use of indicators, of which the bacterial indicators –*E. coli* or faecal coliforms are most widely used. The following section assesses the suitability of "indicator bacteria" and other microbial indicators in wastewater and receiving water.

4.1 BACTERIAL INDICATORS

As bacterial indicators are most commonly used to indicate faecal pollution, it is worthwhile assessing their suitability as indicators of viral presence. The properties and stability of bacteria and viruses in the environment are very different. Enteric viruses tolerate environmental stressors and may survive in the environment for several weeks or even months, whereas enteric bacteria tend to die off within a few days. Epidemiological evidence that viruses are a risk to health in shellfish has been established from outbreaks of gastroenteritis, but similar evidence of risk from viral contamination of recreational water has been more difficult to establish. Occurrence of viruses in recreational water may also be sporadic, making them difficult to detect. Shellfish bioaccumulate micro-organisms reducing their temporal variations, making them easier to detect. Much literature on the reliability of bacterial indicators therefore relates to illness from shellfish consumption.

Although national and international water quality regulations are framed around *E. coli* or faecal coliforms, in a survey of 160 wild and cultured molluscs, *E. coli* criteria did not offer much protection against viruses in either the wild or cultured mollusks (Rose & Gerba, 1991). Viral outbreaks have been reported by Lees (2000) and Rehnstam-Holm & Hernroth (2005) despite compliance with the bacterial standards for shellfish-growing water or shellfish flesh. Hence, it is not surprising that viral outbreaks from shellfish consumption occur despite *E. coli* compliance. The failure of bacterial indicators to protect health has led to the recognition that bacterial indicators are inadequate to assess viral contamination and that a more suitable indicator is required (Romalde et al., 2002; Doré et al., 2000; Hernroth et al., 2002; Lees 2000).

4.2 BACTERIOPHAGES AS INDICATORS OF ENTERIC VIRUSES

The inadequacies of bacterial indicators in protecting public health has led to the proposal of F-specific RNA bacteriophage (F-RNA bacteriophage) being used as an enteric viral indicator for environmental waters and shellfish (Doré et al., 1998; 2000*)* F-RNA bacteriophage is a bacterial virus, which infects *E. coli*, replicates within the bacterial cell to cause lysis of the bacteria and is then released into the environment. F-RNA bacteriophages originate in human and warm-blooded animal faeces (e.g. pigs, sheep, cattle, birds) and

[2] The requirement for notification is often the determining factor (Goblick Pers. Comm)

generally occur in high numbers in sewage and polluted waters (Calci et al., 1998; Havelaar et al., 1993) F-RNA bacteriophages indicate both animal and human faecal pollution, rather than human enteric viruses only, and so F-RNA bacteriophage data should be used with caution in relation to enteric virus contamination.

Havelaar et al. (1993) found a good correlation between enterovirus, reovirus and F-RNA bacteriophage levels in the Rhine River. However, Havelaar et al. (1993) stated that this relationship should not be applied directly to other settings. This is especially important if it were to be applied to marine waters. The importance of determining relationships in other geographic locations is highlighted in the difference in observed relationships between F-RNA phage and enteric virus. A clear correlation was observed between the occurrence of F-RNA phages and enteric viruses in shellfish and their growing waters in northern Europe and the US (Chung et al., 1998; Myrmel et al., 2004; Doré et al., 2000; Formiga-Cruz et al., 2003). Recommendations were made for F-RNA to be used as an indicator of viral contamination (Doré et al., 1998) However, not all research supports this relationship with negligible correlation between the presence of enteric viruses and F-RNA phages, *Bacteroides fragilis* phages or somatic coliphages in mussels found in southern Europe (Spain, France, Italy) (Croci et al., 2000; Moussec et al., 2001). The relationship between the occurrence of F-RNA bacteriophage and enteric viruses was investigated in a 2004-2006 New Zealand study (Greening, 2007). F-RNA bacteriophage were detected in 211/318 (66.3%) shellfish samples, but their presence was not clearly associated with presence of enteric viruses (adenovirus, norovirus, enterovirus) In both Southern Europe and New Zealand a correlation between F-RNA bacteriophage and enteric virus occurrence in environmental waters only occurred where waters are heavily contaminated with sewage.

Another common bacteriophage proposed as an indicator is somatic coliphage. Again there are conflicting results. A poor correlation was found between a range of enteric viruses (adenovirus, rotavirus, enterovirus HAV and Torque teno virus and somatic coliphages in wastewater, receiving water or indicators (Carducci et al., 2006). Conversely, Mocé-Llivinia et al. (2005) found somatic coliphages the best indicator of culturable enterovirus in receiving waters (Table 3).

4.3 OTHER VIRAL INDICATORS FOR WATER QUALITY

Ideally, a human virus would be the best 'indicator' of viral pollution. Many enteroviruses are easily grown in cell culture, and have the advantage that infectivity can also be assessed within a short timeframe. Methods for virus quantitation in environmental samples have mainly focused on enteroviruses as the indicator of viral contamination. However, enteroviruses do not appear to be prevalent in New Zealand environmental waters and so my not be appropriate as indicators of general viral contamination.

Puig et al. (1994) proposed that human adenoviruses detected by PCR could be used as an 'index' of human viral contamination because they can be detected in many environments where enteric viruses are present, whereas traditional bacterial indicators such as faecal coliforms may be absent. Adenoviruses are considered more stable in the environment than enteroviruses, and are more resistant to environmental stressors than many of the enteric viruses. They were proposed as the sentinel virus for UV irradiation of wastewater effluent (USEPA, 2003) The 18-month European shellfish study also endorsed the possible use of human adenovirus detection by PCR as a molecular index of viral contamination in shellfish (Formiga-Cruz et al. 2002). The EU Virobathe study (Virobathe, 2009) has included adenovirus prevalence in recreational waters along with norovirus, which is another proposed virus indicator owing to its inclusion on the 1998 EPA Contaminant List (Schaub et al., 2000). However, the usefulness of PCR-based methods is limited in that they cannot distinguish between infectious and inactivated virus particles especially where a large proportion of the viruses are expected to be non-infectious due to wastewater disinfection or prolonged environmental exposure.

Combinations of bacterial and viral indicators were used to assess the risk to recreational water users and consumers of wild shellfish near the WWTP discharge at Mangere in Auckland. The study compared indicators (*Clostridium perfringens* spores, *E. coli*, enterococci, F-RNA phage) with viral pathogens (adenovirus and enterovirus), but concluded that there were no suitable surrogate organisms to monitor virus removal (DRG, 2002).

5 CONCLUSION

At this time, there are no suitable international enteric virus guidelines, standards or regulations to protect public health when using recreational water, or in shellfish-growing waters. The water quality of 0.04 enteric viruses/L is a hypothetical example of the potential risk and it has a significant drawback owing to the inability to detect and measure every enteric virus within this concentration limit. An enteric virus water quality standard is impractical to implement.

It is recognised that there no generic indicators for enteric viruses and that *E. coli* is not conservative enough as they are much less persistent in the receiving environment than enteric viruses. In certain situations there appears to be a relationship between specific viruses and bacteriophages, but such relationships are likely to vary geographically and temporally. Adenovirus may be too conservative as they survive for a long time in the environment and may be indicative of a previous contamination event, rather than indicative of a risk of infection from consumption of shellfish. Use of PCR based methods also limits ability to determine if viruses are infective.

In the absence of a preferred viral indicator, it is appropriate to use a combination of tools for managing water quality. These include supporting the current water quality guidelines which include monitoring and sanitary surveys, with improved knowledge of viral inputs and outputs from different WWTP and risk assessment.

The QMRA was a useful tool to determine an appropriate level of treatment for the Mangere WWTP. The output from the QMRA was to determine an acceptable concentration of viruses in the WWTP effluent, taking into account the zone required for dilution of viruses before contact with shellfish gathering areas – a buffer zone, as used in the USA. The 4-log virus removal criterion was based on a pragmatic approach between managing risk and the practicalities of installing and operating a WWTP for Mangere. Analysis of the field inputs confirm that it was site-dependent and therefore it should not be applied generically to other areas. However, the principles of risk management could be applied elsewhere.

An efficacious approach would be to develop guidelines establishing a tiered approach to risk assessment, which would account for the treatment process and receiving environment, to compliment the development in analytical techniques and support the current guidelines. Application of a blanket 4-log removal requirement will be over precautionary and unnecessarily expensive in most situations and a detailed QMRA would be prohibitively expensive for small communities.

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REFERENCES

Asano, T. Leong, L.Y.C., Rigby, M.G., Sakaji, R.H. (1992) 'Evaluation of the California wastewater reclamation criteria using enteric virus monitoring data' *Water Science and Technology*, 26, 7-8, 1513-1542.

Calci, K.R. Burkhardt, W. Watkins, W.D., Rippey, S.R. (1998) 'Occurrence of male-specific bacteriophage in feral and domestic animal wastes, human feces, and human-associated wastewaters' *Applied Environmental Microbiology*, 64, 12, 5027-9.

Carducci, A., Verani, M.; Battistini, R., Pizzi, F., Rovini, E., Andreoli, E., Casini, B. (2006) 'Epidemiological surveillance of human enteric viruses by monitoring of different environmental matrices' *Water Science and Technology*, 54, 3, 239-244.

Chung, H., Jaykus, L.A., Lovelace, G., Sobsey, M.D. (1998) 'Bacteriophages and bacteria as indicators of enteric viruses in oysters and their harvest waters' *Water Science and Technology*, 38, 37-44.

Crabtree, K. D., C. P. Gerba, et al. (1997) 'Waterborne adenovirus: a risk assessment' *Water Science and Technology,* 35, 11-12, 1-6.

Craig, D.L., Fallowfield, H.J., Cromar, N.J. (2002) 'Comparison of decay rates of faecal indicator organisms in recreational coastal water and sediment' *Water Science and Technology. Water Supply*, 2, 3, 131-138.

Crance, J.M., Gantzer, C., Schwartzbrod, L., Deloince, R. (1998) Effect of temperature on the survival of Hepatitis A virus and its capsidal antigen in synthetic seawater. *Environmental Toxicology Water Quality*, 13, 1, 89-92.

Croci, L., De Medici, D., Scalfaro, C., Fiore, A., Divizia, M., Donia, D. (2000) 'Determination of enteroviruses, hepatitis A virus, bacteriophages and Escherichia coli in Adriatic Sea mussels' *Journal of Applied Microbiology*, 88, 293-298.

Dahling, D.R., Saferman, R.S., Wright, B.A. (1989) 'Isolation of enterovirus and reovirus from sewage and treated effluents in selected Puerto Rican communities' *Applied Environmental Microbiology*, 55, 2, 503-506.

Doré, W.J., Henshilwood, K., Lees, D.N. (1998) 'The development of management strategies for control of virological quality in oysters' *Water Science and Technology*, 38, 12, 29-35.

Doré WJ; Henshilwood K; Lees DN. (2000) 'Evaluation of F-specific RNA bacteriophage as a candidate human enteric virus indicator for bivalve molluscan shellfish' *Applied Environmental Microbiology*, 66, 4, 1280-1285.

DRG. (2002) 'Pilot Study investigations, Surrogate Study results and recommendations' Disinfection Review Group report to Watercare Services Ltd.

Formiga-Cruz, M., Tofino-Quesada, G., Bofill-Mas, S., Lees, D.N., Henshilwood, K., Allard, A.K., Conden-Hansson, A.C., Hernroth, B.E., Vantarakis, A., Tsibouxi, A., Papapetropoulou, M., Furones, M.D., Girones, R. (2002) 'Distribution of human virus contamination in shellfish from different growing areas in Greece, Spain, Sweden and the United Kingdom' *Applied Microbiology*, 68, 12, 5990-5998.

Formiga-Cruz, M., Allard, A.K., Conden-Hansson, A.C., Henshilwood, K., Hernroth, B.E., Jofre, J., Lees, D.N., Lucena, F., Papapetropoulou, M., Rangdale, R.E., Tsibouxi, A., Vantarakis, A., Girones, R. (2003) 'Evaluation of potential indicators of viral contamination in shellfish and their applicability to diverse geographical areas' *Applied Environmental Microbiology*, 69, 3, 1556-1563.

Gantzer, C., Dubois, Ė., Crance, J-M., Billaudel, S., Kopecka, H., Schwartzbrod, L., Pommepuy, M., Le Guyader F. (1998) 'Devenir des entériques en mer influence des facteurs environnementaux' *Ocean. Acta* 21, 6, 983-992.

Gerba, C. (1984) 'Applied and theoretical aspects of virus adsorption to surfaces' *Advanced Applied Microbiology*, 30, 133-168.

Goblick, G. Consumer Safety Officer, U.S. FDA, Maryland. pers. comm. (2008)

Greening, G.E., Hewitt, J., Lewis, G.D. (2000) 'Seasonal presence of enteric viruses and F-RNA bacteriophage in New Zealand Sewage'. Presented at Water 2000 International Water Association Conference, Paris, 2000

Greening http://www.esr.cri.New Zealand/SiteCollectionDocuments/ESR/PDF/FoodSafety/FRSTShellfish-Report-Final-Aug07.pdf. (2007).

Havelaar A. H., van Olphen, M., Drost, Y.C. (1993) 'F-specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water' *Applied Environmental Microbiology*, 59, 9, 2956-2962.

Hernroth, B.E., Conden-Hansson, A-C., Rehnstam-Holm, A-S., Girones, R., Allard, A.K. (2002) 'Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, Mytilus edulis: The first Scandinavian report' *Applied Environmental Microbiology*, 68, 9, 4523-4533.

Hewitt, J. and Greening, G.E. (2005) 'Removal of noroviruses, adenoviruses and enteroviruses in wastewater by New Zealand sewage treatment plants'. Poster. Health-related Water Microbiology Meeting, Swansea, UK, 9-14 September 2005.

Hurst, C.J., McClellan, K.A., Benton, W.H. (1988) 'Comparison of cytopathogenicity, immunofluorescence and in situ DNA hybridization as methods for the detection of adenovirus' *Water Research*, 22, 1547-1552.

Irving, L.G and Smith, F.A. (1981) 'One-year study of enteroviruses, adenoviruses and reoviruses isolated from effluent of an activated sludge purification plant' *Applied Environmental Microbiology*, 41, 51-59.

Jacangelo, J.G., Loughran, P., Petrik, B., Simpson, D., McIlroy, C. (2003) 'Removal of enteric viruses and selected microbial indicators by UV irradiation of secondary effluent' *Water Science and Technology*, 47, 9, 193-198.

Kohn, M.A., Farley, T.A., Ando, T., Curtis, M., Wilson, S.A., Jin, Q., Monroe, S.S., Baron, R.C., McFarland, L.M., Glass, R.I. (1993) 'An outbreak of Norwalk virus gastroenteritis associated with eating raw oysters: Implications for maintaining safe oyster beds' *JAMA* 273, 6, 466-471.

Krikelis, V., Spyrou, N., Markoulatos, P., Serie, C. (1985) 'Seasonal distribution of enteroviruses and adenoviruses in domestic sewage' *Canadian Journal of Microbiology*, 31, 24-25.

Lees DN. (2000) Viruses and bivalve shellfish International *Journal of Food Microbiology*, 59, 1-2, 81-116.

Leonard, M.M., Garrett, N., Bourke, M., Gilson, M. (2003) 'Removal of microbial pathogens and indicators from the wastewater stream' New Zealand Water and Waste Conference, Auckland.

Lewis, G.D., Austin, F.J., Loutit, M.W., Sharples, K. (1986) 'Enterovirus removal from sewage The effectiveness of four different treatment plants' *Water Research*, 20, 10, 1291-1297.

Ministry for the Environment (2003) Microbial water quality guidelines for marine and freshwater recreational areas, Ministry for the Environment, Wellington.

Mocé-Llivinia, L., Lucena, F., Jofre, J. (2005) 'Enteroviruses and bacteriophages in bathing waters' *Applied Environmental Microbiology*, 71, 11, 6838-6844.

Moore, B.E., Camann, D.E., Turk, C.A., Sorber, C.A. (1988) 'Microbial characterization of municipal wastewater at a spray irrigation site: the Lubbock infection surveillance study' *Journal of Water Pollution Control Federation*, 60, 7, 1222-1230.

Moussec, L., Le Guyader, F., Pelletier, D., Haugarreau, L., Caprais, M.-P., Pommepuy, M. (2001) 'Validity of Escherichia coli, enteroviruses, and F-specific RNA bacteriophages as indicators of viral shellfish contamination' *Journal of Shellfish Research*, 20, 1223-1227.

Myrmel, M., Berg, E.M.M., Rimstad, E., Grinde B. (2004) 'Detection of enteric viruses in shellfish from the Norwegian coast' *Applied Environmental Microbiology*, 70, 5, 2678-2684.

Nasser, A.M., Zaruk, N., Tenenbaum, L., Netzan, Y. (2003) 'Comparative survival of Cryptosporidium, coxsackievirus A9 and Escherichia coli in stream, brackish and sea waters' *Water Science and Technology*, 47, 3, 91-96.

Nasser, A.M., Zev, L., Fattal, B. (1994) 'Removal of indicator microorganisms and enteroviruses from wastewater in a single cell stabilisation pond' *International Journal of Environmental Health Research*, 4, 7-16.

Puig, S., Jofre, J., Lucena, F., Allard, A., Wadell, G., Girones R. (1994) 'Detection of adenoviruses and enteroviruses in polluted waters by nested PCR amplification' *Applied Environmental Microbiology*, 60, 8, 2963-2970.

Rehnstam-Holm, A-S., and Hernroth, B. (2005) 'Shellfish and public health: a Swedish perspective' *Ambio,* 34, 2, 139-144.

Resource Management Act, 1991

Romalde, J.L., Area, E., Sánchez, G., Ribao, C., Torrado, I., Abad, X., Pintó, R.M., Barja, J.L., Bosch A. (2002) 'Prevalence of enterovirus and hepatitis A virus in bivalve molluscs from Galicia (NW Spain): inadequacy of the EU standards of microbiological quality' *International Journal of Food Microbiology*, 25, 1-2, 119-130.

Rose, J.B. and Gerba, C.P. (1991) 'Use of risk assessment for development of microbial standards' *Water Science and Technology*, 34, 2, 29-34.

Rose, J. B. & M. D. Sobsey (1993). 'Quantitative risk assessment for viral contamination of shellfish and coastal waters' *Journal of Food Protection* 56, 12, 1043-1050.

Rose, J.B., Dickson, L.J., Farrah, S.R., Carnahan, R.P. (1996) 'Removal of pathogenic and indicator microorganisms by a full-scale water reclamation facility' *Water Research* 30, 11, 2785-2797.

Salter, H.E., Boyle, L., Ouki, S.K., Quarmby, J., Williams, S.C. (1999) 'The performance of tertiary lagoons in the United Kingdom:1' *Water Research*, 33, 18, 3775-3781.

Schaub, S.A. and Oshiro, R.K. (2000) 'Public health concerns about caliciviruses as waterborne contaminants' *Journal of Infection and Disease,* 181, Supplement 2, S374-S380.

Sedmark, G., Bina, D., MacDonald, J., Couillard, L. (2005) 'Nine-year study of the occurrence of culturable viruses in source water for two drinking water treatment plants and the influent and effluent of a wastewater treatment plant in Milwaukee, Wisconsin (August 1994 thorough July 2003)' *Applied Environmental Microbiology*, 71, 2, 1042-1050.

Simpson, D., Jacangelo, J., Loughran, P., McIlroy, C. (2003) 'Investigation of potential surrogate organisms and public health risk in UV irradiated secondary effluent', *Water Science and Technology*, 47, 9, 37-43.

Teunis, P.F.M., Moe, C.L., Liu, P., Miller, S.E., Lindesmith, L., Baric, R.S., Le Pendu, J., Calderon, R.L. (2008) 'Norwalk virus: how infectious is it?' *Journal of Medical Virology*, 80, 1468-1476.

Tree, J.A., Adams, M.R., Lees, D.N. (2003) 'Chlorination of indicator bacteria and viruses in primary sewage effluent' *Applied Environmental Microbiology*, 69, 4, 2038-2043.

USEPA. UV disinfection guide. Draft February 2003.

Virobathe (2009) http://www.virobathe.org/

Wait, D.A. & Sobsey, M.D. (2001) 'Comparative survival of enteric viruses and bacteria in Atlantic Ocean seawater' *Water Science and Technology*, 43, 12, 139-142.

Wetz JJ; Lipp EK; Griffin DW; Lukasik J; Wait D; Sobsey MD; Scott TM; Rose JB. (2004) 'Presence, infectivity, and stability of enteric viruses in seawater: relationship to marine water quality in the Florida Keys' *Marine Pollution Bulletin*, 48, 7-8, 698-704.

Wyn-Jones pers. comm. 2008, Virobathe Vice Co-ordinator, Aberystwyth University