

# AGRICULTURAL REUSE OF POND EFFLUENT AND QUANTITATIVE MICROBIAL RISK ASSESSMENT

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## ABSTRACT

Pond effluent could be an important alternative water source for agricultural irrigation due to its low cost, good pathogen removal rate and nutrient richness, and reliability of supply. The microbial hazards are the primary concern of the public health protection requirements for the use of recycled water. Quantitative microbial risk assessment (QMRA) is promoted in the Australian water recycling guidelines for managing risk of reuse schemes. Using rotavirus as a reference pathogen in characterizing human health hazard, this paper effectively demonstrates how to apply QMRA analysis for decision making in health risk assessment for a water recycling scheme.

**Keywords:** pond effluent; agricultural reuse; quantitative microbial risk assessment; dose-response model

## INTRODUCTION

As of 2012, about 1200 to 1300 Wastewater Treatment Plants (WTP) operated in Australia of which about 600 are pond systems Geoscience Australia (2014). We estimated that pond system WTPs serve at least 600,000 Australians residing in small, often remote communities. Currently, more than 600 different recycled water schemes operate in Australia Radcliffe (2010). Historically, agricultural irrigation represented the largest use of recycled water in the country. However, the fraction of recycled water used in agriculture decreased from 66% to 29% (thought still marginally the single largest proportion) over the period 2004 to 2009 Chen et al. (2013); Radcliffe (2010). Figure 1 depicts a typical pond-based wastewater treatment system in which biochemical oxygen demand (BOD) is mainly removed in the anaerobic pond. The facultative pond functions partially for BOD removal (the lower part of the pond) and partially for pathogen removal (in the upper part of the water body and by sedimentation)

Mara (2004); Shilton (2005). The maturation ponds primarily function to remove pathogen through complex bio-chemical and physical processes depending on factors such as sun-light intensity, pH, temperature, algae populations, sedimentation, predation, etc. Pond wastewater treatment systems can typically achieve 1 to 4 log reductions in pathogen concentrations (i.e., 90 – 99.99% reduction/removal) but is relatively poor at nutrient removal Mara (2004); NRMCC et al. (2006); Shilton (2005). The majority of Australia belongs to tropical or warm temperate regions. It is well known that pond systems can operate effectively throughout the year in these regions (Mara, 2004); Shilton (2005). In contrast to a conventional electric-mechanical driven (e.g., an activated sludge process based) wastewater treatment plant, a properly designed and operated pond system provides an alternative choice of low cost, less high-tech dependent, and chemical-free solution for domestic wastewater treatment for many small communities in vast rural regions. Because the amount of wastewater produced is usually proportional to the water consumption volumes of a population, pond effluent may be a more reliable source of water than rainfall-dependent sources Chen et al. (2013); Scheierling et al. (2010). Therefore, potentially, pond effluent can be an appropriate and important alternative water source for agricultural irrigation in Australia due to its low cost, good pathogen removal rate and nutrient richness, and reliability of supply.

The potential risks for the use of recycled water (e.g., pond effluent) include the environmental impacts (such as elevated levels of dissolved solids, changes in water chemistry, heavy metal accumulation, etc.) and human health risks. While the environmental protection concern could be as important, this paper is restricted to quantitative microbial risk assessment (QMRA) Haas et al. (2014) for protection of human health. A multitude of diseases are associated with exposure to wastewater. These are caused by exposure to pathogens (bacteria, viruses, protozoa and

helminths) and chemicals as well as potentially toxic blooms of cyanobacteria. Of the public health protection requirements, the microbial hazard is the

primary concern for the use of recycled water MEDAWARE (2005); NRMMC et al. (2006); WHO (2006).

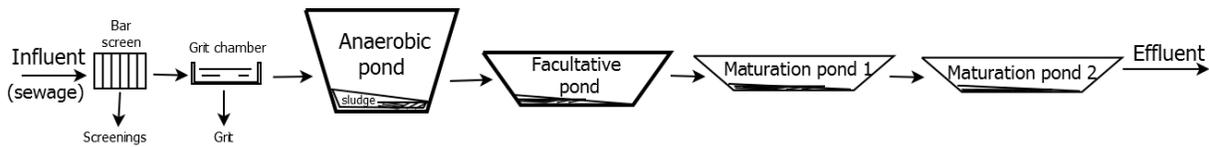


Figure 1: A schematic showing a typical waste stabilization pond system (the pond system)

Recycled water microbial risks are now assessed by reference to a tolerable additional disease burden measured by a disability-adjusted life year (DALY) loss per person per year (pppy) NRMMC et al. (2006). The health-based target is a benchmark or goal-post which has been set up as that  $10^{-6}$  DALYs pppy is not exceeded and this has to be met by each recycled water scheme NRMMC et al. (2006); WHO (2006). This approach is seen in both the 2006 WHO *Guidelines for the safe use of wastewater, excreta and greywater, Volume 2* and the 2006 *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase1)* NRMMC et al. (2006); WHO (2006). However, it is a challenge for recycled water management regulators and operators to implement these guidelines, because the health-based target of  $10^{-6}$  DALYs pppy needs to be translated into log removal requirement for the wastewater treatment processes. This is complicated by different end-use scenarios MEDAWARE (2005); Scheierling et al. (2010). QMRA is recommended by these guidelines for filling the gaps in water recycling risk management NRMMC et al. (2006); WHO (2006). Setting health-based targets for all pathogens present in recycled wastewater would be impractical as it would require vast sets of pathogen concentration data, dose-response data and epidemiological data for the burden of disease in the population. Rotavirus has been widely used as a reference pathogen in characterizing the health hazard in agricultural reuse of treated wastewater in QMRA analysis because rotaviruses pose a major threat of viral gastroenteritis worldwide – rotavirus is detected in large numbers in sewage, and has a low infectious dose Haas et al. (2014); NRMMC et al. (2006); Teunis and Havelaar (2000). Due to the mathematical complexity in the exact Beta-Poisson dose-response model, an approximate model has been proposed to facilitate its applications in QMRA Furumoto and Mickey (1967); Haas et al. (2014). However, the usage of the approximate Beta-Poisson model for characterizing the rotavirus dose-response relation has caused some discussions on the accuracy of the approximation Haas et al. (2014); Teunis and Havelaar (2000).

This paper demonstrates how to apply QMRA analysis for decision making in health risk assessment for a hypothetical water recycling scheme against the health-based target criterion specified in the guidelines using rotavirus as a reference pathogen to characterize the health hazard. Solutions and evidence are provided to show how to apply the rotavirus dose-response model properly in QMRA analysis.

#### METHODOLOGY AND PROCESS

The QMRA approach is a four-step process comprising (i) hazard identification; (ii) exposure assessment; (iii) dose-response analysis; and (iv) risk characterization.

DALY is defined as  $DALYs = YLL$  (years of life lost) +  $YLD$  (years lived with a disability or illness) NRMMC et al. (2006). For example, using Australian data with this DALYs formula, infection with rotavirus causes an estimated 0.013 DALY loss per case NRMMC et al. (2006). The tolerable probability of infection pppy can be calculated as follows NRMMC et al. (2006).

$$\text{Tolerable disease risk pppy} = \frac{\text{Tolerable DALY loss pppy}}{\text{DALY loss per case of disease}} \quad (1)$$

$$\text{Tolerable probability of infection pppy} = \frac{\text{Tolerable disease risk pppy}}{\text{Disease-to-infection ratio}} \quad (2)$$

The disease-to-infection ratio for rotavirus is 0.05 NRMMC et al. (2006). Therefore, from Eqs. (1) and (2) the tolerable probability of infection pppy = 0.00154 is obtained as shown in the right-bottom corner of Figure 2. For public health protection purpose, a water recycling scheme should meet this criterion (i.e., the estimated probability of pppy from a water recycling scheme should not exceed 0.00154) through risk assessment. The left-hand part of Figure 2 shows this QMRA process schematically.

The core part of QMRA framework is the dose-response analysis which models the mathematical characterization of the relationship between the

dose administered and the probability of adverse effect (typically, the probability of infection) in the exposed population. Let  $F_1(d)$  denote the probability of infection of a single exposure event.  $F_1(d)$  can be evaluated using the popular Beta-Poisson dose response model which takes the form

$$F_1(d) = 1 - {}_2F_1(\alpha, \alpha + \beta, -d), \quad (3)$$

where  ${}_2F_1(\alpha, \alpha + \beta, -d)$  is the Kummer confluent hypergeometric function with model parameters  $\alpha, \beta$  and  $d$  is the effective dose (e.g., number of rotavirus per 100 mL). Since no closed form exists for  ${}_2F_1(\alpha, \alpha + \beta, -d)$ , only numeric approximation solutions or asymptotic solutions can be obtained for Eq. (3) Butler and Wood (2002). In their original

paper, Furumoto and Mickey (1967) derived the simple, attractive approximation Beta-Poisson dose-response formula to facilitate the application of Beta-Poisson dose-response models:

$$F_1(d) = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha} \quad (4)$$

Eq. (4) requires that  $\alpha \ll \beta$  and  $\beta \gg 1$ , given the effective dose levels  $d$ , for valid application Haas et al. (2014); Teunis and Havelaar (2000). The dose-response relation for rotavirus is a typical case in which the approximate Beta-Poisson model formula Eq. (4) fails because  $\hat{d} = 0.253$  and  $\hat{\beta} = 0.426$ , or  $\hat{d} = 0.253$  and  $N_{50} = 6.17$  ( $N_{50}$  is the the median effective dose, i.e., the effective dose level  $d$  when  $F_1(d) = 0.5$  based on Eqs. (4)).

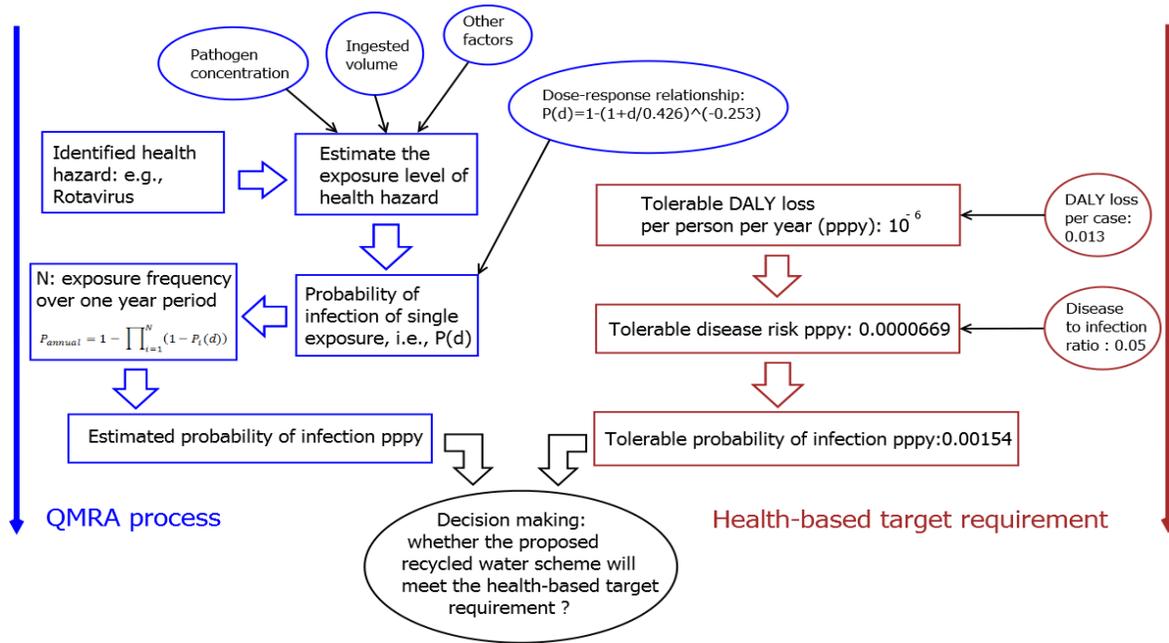


Figure 2: A schematic showing how QMRA is applied in a recycled water end-use scenario.

With the probability of infection of a single exposure event estimated from Eq. (4), the estimated probability of infection pppy can be calculated by formula

$$P_{\text{annual}} = 1 - \prod_{i=1}^N (1 - F_1(d)), \quad (5)$$

where  $N$  is the number of exposure events over a one-year period (i.e., the annual consumption frequency). The approval of a water recycling scheme requires  $P_{\text{annual}} \leq 0.00154$  using rotavirus

as the measure of public health hazard. Therefore, it is vital to have a good estimation of  $F_1(d)$  by a correct application of Eq. (4). Figure 3 shows that the lack of accuracy in approximating the exact Beta-Poisson model using Eq. (4) is substantial: the median effective dose  $N_{50} = 6.17$  (the approximate Beta-Poisson model result) is much higher than the correct answer 2.54 (the exact Beta-Poisson model result). Therefore, a piece-wise approximate Beta-Poisson model specification is proposed in this paper to improve the original approximation.

## RESULTS/ OUTCOMES

Our research proposed a piece-wise approximate Beta-Poisson model as  $P_i(d) = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha}$ , where  $\hat{\alpha} = 0.6554$  and  $\hat{\beta} = 1.294$  when  $d \leq 2$ ;  $\hat{\alpha} = 0.1707$  and  $\hat{\beta} = 0.02413$  when  $d > 2$ . This piece-wise approximate model specification gives a very good approximation to the exact Beta-Poisson model for characterizing the dose-response relation for rotavirus (Figure 4).

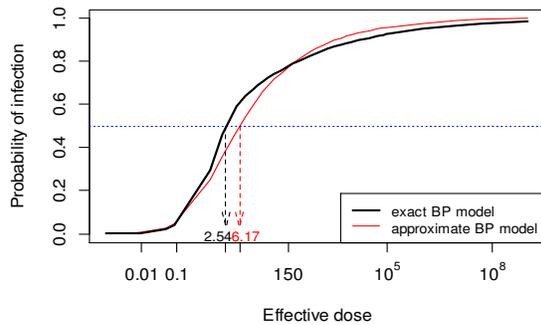


Figure 3: Comparison of the estimated median effective dose ( $N_{50}$ ) of rotavirus: exact Beta-Poisson model versus approximate Beta-Poisson model

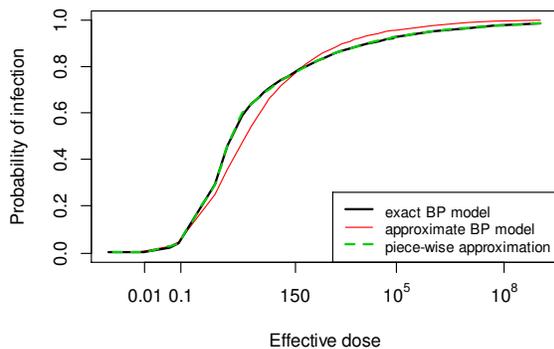


Figure 4: The piece-wise approximate Beta-Poisson dose-response model represented by the bold dashed lines which almost perfectly match the bold solid line (the exact model).

For a hypothetical water recycling scheme, we adopted Hamilton's formula:  $d = BMCV \exp(-kt)$  Hamilton et al. (2006) for determining the hazard exposure level. In the formula, B = human body mass in kg; M = daily consumption of lettuce (in grams) per kg of body mass per person; c = rotavirus concentration in pfu per mL (pfu = plaque-forming units) of the pond effluent for irrigation; V = volume of water caught by 1 gram of lettuce; and

$\exp(-kt)$  is a first-order exponential decay model used to describe viral inactivation on surface of the plant with k being the decay coefficient and t the number of days elapsed since the last irrigation (i.e., withholding period). Therefore, the effective dose  $d$  is defined as the exposure level in terms of number of virus ingested in one incidence. The probability of infection of a single event (per person) then can be calculated using the piece-wise approximate Beta-Poisson model as described above. The annual consumption frequency N is also required for the calculation of probability of infection pppy. Given this end-use scenario setting, the QMRA process as schematically depicted in Figure 2 can be demonstrated as follows.

### A deterministic approach example (all model parameters are assumed to be some fixed values)

Let us assume a scenario case of lettuce eaten raw with the following assumptions: B = 61 kg; M = 0.102 g/kg/day; c = 0.15 pfu/L = 0.00015 pfu/mL; V = 0.108 mL/g; k = 1.07 and t = 1 day. Annual consumption frequency N = 52 (e.g., once every week).

Therefore, in this particular case, the identified health hazard is rotavirus (Step 1 of QMRA process); The exposure level can then be calculated as

$d = 61 \cdot 0.102 \cdot 0.00015 \cdot 0.108 \cdot \exp(-1.07 \cdot 1) = 0.000034574$ , where  $\exp(1) \equiv e^1 \approx 2.718$  (Step 2 of QMRA process). The probability of infection of a single exposure event can be calculated as

$$P_i(d) = 1 - \left(1 + \frac{0.000034574}{1.294}\right)^{-0.6554} = 0.00001751$$

(Step 3 of QMRA process). Finally, the probability of infection pppy is calculated using Eq. (5) as

$$P_{\text{annual}} = 1 - (1 - 0.00001751)^{52} = 0.0009101$$

(Step 4 of QMRA process). Since 0.0009101 is less than 0.00154, the tolerable probability of infection pppy, this water recycling scheme may be approved. However, if the annual consumption frequency is changed to N = 183 (e.g., one consumption every other day), the resulting probability of infection pppy becomes

$$P_{\text{annual}} = 1 - (1 - 0.00001751)^{183} = 0.003199$$

With this increase in consumption frequency, the human health risk increased as well to such an extent that the tolerable health-based target criterion 0.00154 is no longer met. Therefore, a decision would be made to reject the proposed water recycling scheme.

The above example exemplified that making decisions on accepting a water recycling scheme based on a single value of health risk assessment result is questionable. A more realistic approach is to assume some appropriate statistical distributions for the model parameters based on our prior knowledge. This approach is more difficult to implement due to its stochastic nature and demand

for intensive computation. Computer software such as the statistical package R R Development Core Team (2014) or the Microsoft Excel based risk assessment package @Risk Palisade Corporation (2014) may be employed to perform the analysis through Monte Carlo simulation.

**A probabilistic approach example (all model parameters are assumed to follow some statistical distributions)**

We adopted a scenario case of lettuce eaten raw as detailed in Hamilton (2006) which has the following assumptions:  
 $B \sim \text{lognormal}(\text{mean}=61.429, \text{sd} = 13.362)$  kg;  
 $M = 0.102$  g/kg/day;  $c \sim \text{lognormal}(\text{mean}=0.15, \text{sd} = 0.63)$  pfu/L;  $V \sim \text{normal}(\text{mean} = 0.108, \text{sd}=0.019)$  mL/g (truncated at 0);  $k \sim \text{normal}(\text{mean}=1.07, \text{sd}=0.07)$  (truncated at 0) and  $t = 1$  day or  $t = 2$  days. Annual consumption frequency  $N = 365$  (e.g., once every day) or  $N = 183$  (one consumption every other day), or  $N = 52$  (e.g., once every week). As shown in Table 1, four end-use scenarios are assumed and the estimates of the probability of infection pppy for each scenario are presented. The corresponding graphic outcomes are shown in Figure 5. Note that, unlike the deterministic

approach solution, the resulting probability of infection pppy is now in a distributional form which is a better representation of reality. It is then up to the decision maker to decide if the mean or median value of the estimated probability of infection (as a typical estimate), or the 95<sup>th</sup> percentile value (as a conservative estimate) should be used for health risk assessment. For example, Scenario (b) assumes that the population in concern has a consumption frequency of 183 per year and the withholding period is 1 day. Based on Table 1, we know that the health-based target 0.00154 is not met by either median estimate criterion or the 95<sup>th</sup> percentile criterion. On the other hand, in Scenario (d), the same annual consumption frequency is assumed but with a different withholding period which is 2 days. This small change in assumptions results in substantial changes in the final estimates of the probability of infection pppy. The mean and median values are well below the health-based target value 0.00154; even the estimated 95<sup>th</sup> percentile value 0.00169 only marginally exceeded the health-based target value. Therefore, QMRA is able to provide the valuable quantitative evidence for decision making in the water recycling scheme approval process.

Table 1: Health risk assessment results based on Hamilton's exposure formula Hamilton et al.(2006):  $a = BMCVexp(-kt)$ , with different annual consumption frequency (N) and elapsed days since last irrigation prior to consumption (t): Scenario (a)  $N = 365, t=1$ ; Scenario (b)  $N = 183, t=1$ ; Scenario (c)  $N = 52, t=1$ ; Scenario (d)  $N = 183, t=2$ .

Probability of infection per person per year	Mean	Median	95 <sup>th</sup> percentile	Note (withholding period)
Scenario (a)	0.00648	0.00618	0.00894	Daily consumption (t = 1 day)
Scenario (b)	0.00324	0.00304	0.00480	One consumption every other day (t = 1 day)
Scenario (c)	0.000936	0.000801	0.00187	Weekly consumption (t = 1 day)
Scenario (d)	0.00110	0.00104	0.00169	One consumption every other day (t = 2 days)

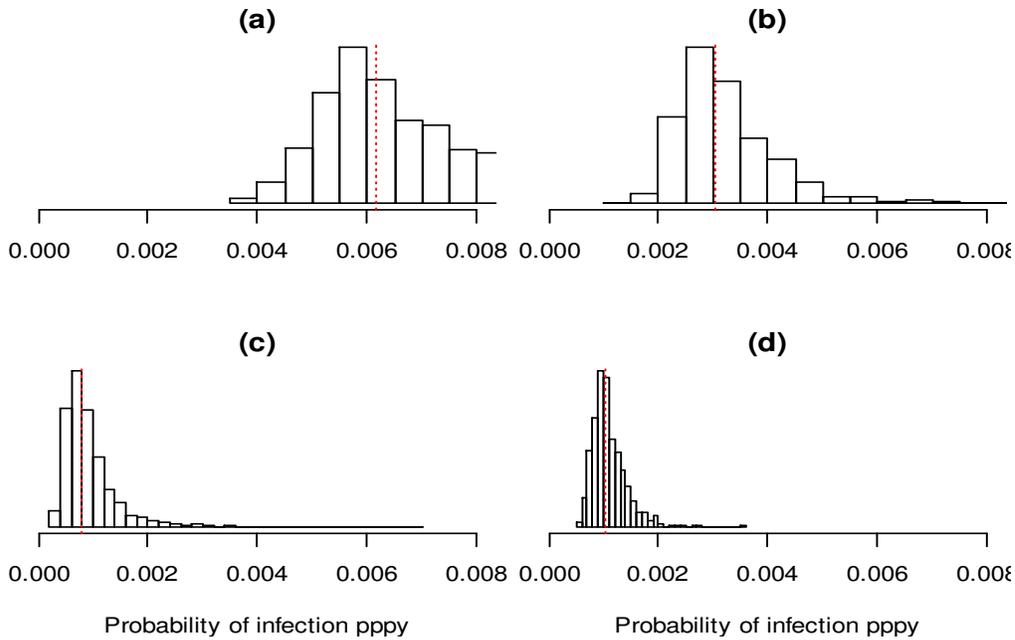


Figure 5: Histograms of the distributions of probability of infection pppy given different end use scenarios: (a) consumption frequency  $N=365$  (daily) and time-lag  $t=1$  day; (b)  $N = 183$  (every other day),  $t=1$ ; (c)  $N = 52$  (weekly),  $t = 1$ ; (d)  $N = 183$  (every other day),  $t = 2$ . Dotted vertical lines (in red colour) indicate the median probability of infection pppy.

### Log removal requirement

As recommended by Australian water recycling guidelines NRMCC et al. (2006), 8000 rotavirus pfu per litre can be used as the default value representing their concentration in raw sewage. Therefore, a pond effluent with 0.15 pfu/L as we have assumed in the above examples is equivalent to a requirement of the log removal value =  $\log_{10}(8000) - \log_{10}(0.15) = 4.73$ . In the probabilistic approach example, we assumed that  $c \sim \text{lognormal}(\text{mean}=0.15, \text{sd} = 0.63)$  pfu/L. Therefore, the corresponding pond log removal value would have a distribution similar to that shown in Figure 6 (median is about 5.5). Since the pond system can typically achieve only 1 to 4 log units of pathogen removal, this implies that in most cases the pond effluent may not be suitable for water recycling schemes without further treatment. Additional health protection measures would be needed in order to meet the health-based target criterion, which could include a longer withholding period, applying different irrigation methods, not irrigating salad crops and vegetables that may be eaten uncooked, and additional clean water washing of the products before consumption, etc.

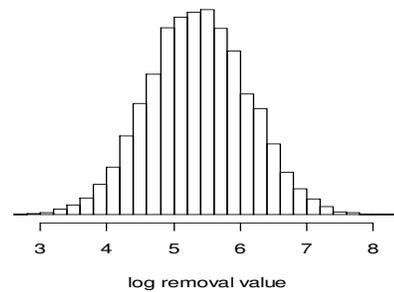


Figure 6: log removal value requirement on pond system based on the model assumptions

### CONCLUSION

There is a huge potential for agricultural reuse of pond effluent in Australia in terms of technical feasibility, health protection, and economic benefit. QMRA is a useful analysis tool widely accepted for health risk assessment in the appraisal of proposed water recycling schemes. A decision can be made

on a proposed water recycling scheme by examining if the estimated probability of infection pppy from the scheme can satisfy the tolerable probability of infection pppy requirement derived from the specified health-base target as illustrated in Figure 2. So far, most of the reported investigations of viral disease burden from treated wastewater irrigation of vegetables eaten raw have shown that a log removal of 1 to 4 units is not sufficient to meet the tolerable probability of infection pppy criterion Hamilton et al. (2006); MEDAWARE (2005). This implies extra health protection measures, such as crop restriction or human exposure control, are needed for implementing such a water recycling scheme. A proper use of the dose-response models in QMRA analysis will ensure a safe and beneficial use of pond effluent in agriculture – wastewater is just too valuable to be wasted!

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