

# Tools for Risk Analysis: Updating the 2006 WHO Guidelines

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## ABSTRACT

This chapter reviews developments since the WHO Guidelines for the safe use of wastewater in agriculture were published in 2006. The six main developments are: the recognition that the tolerable additional disease burden may be too stringent for many developing countries; the benefits of focusing on single-event infection risks as a measure of outbreak potential when evaluating risk acceptability; a more rigorous method for estimating annual risks; the availability of dose-response data for norovirus; the use of QMRA to estimate *Ascaris* infection risks; and a detailed evaluation of pathogen reductions achieved by produce-washing and disinfection. Application of the developments results in more realistic estimates of the pathogen reductions required for the safe use of wastewater in agriculture and consequently permits the use of simpler wastewater treatment processes.

## INTRODUCTION

Since the publication of the 2006 WHO Guidelines for the safe use of treated wastewater in agriculture (WHO, 2006) there have been several pertinent developments in risk analysis techniques and the interpretation of the resulting risks. These include:

- Recognition that a tolerable additional disease burden of  $\leq 10^{-6}$  Disability-Adjusted Life Year (DALY) loss per person per year (pppy) may be too stringent in many developing-country settings and that a DALY loss of  $\leq 10^{-5}$  or even  $\leq 10^{-4}$  pppy may be sufficiently protective of human health (WHO, 2007).
- A persuasive argument for focusing on single-event infection risks as a measure of 'outbreak potential', rather than annual risks alone, when evaluating risk acceptability (Signor and Ashbolt, 2009).
- A more rigorous method for estimating annual risks (Karavarsamis and Hamilton, 2009; see also Benke and Hamilton, 2008).
- The availability of dose-response data for norovirus (Teunis et al., 2008).
- Application of QMRA to estimate *Ascaris* infection risks (Navarro et al., 2009).
- Evaluation of pathogen reductions achieved by produce-washing and disinfection (Amoah et al., 2007).

### LESS STRINGENT TOLERABLE BURDEN OF DISEASE

In *Levels of Protection*, one of the documents in the rolling revision of its drinking-water quality guidelines, WHO (2007) states that, 'in locations or situations where the overall burden of disease from microbial, chemical or radiological exposures by all exposure routes is very high, setting a  $10^{-6}$  DALY [loss] per person per year annual risk from waterborne exposure will have little impact on the overall disease burden. Therefore, setting a less stringent level of acceptable risk, such as  $10^{-5}$  or  $10^{-4}$  DALY [loss] per person per year, from waterborne exposure may be more realistic, yet still consistent with the goal of providing high-quality, safer water and encouraging incremental improvement of water quality.' Following the principles of the Stockholm Framework (Fewtrell and Bartram, 2001), this can be adapted and applied to wastewater use in agriculture.

Thus, for communities with high levels of diarrhoeal disease it is probably unrealistic to set a tolerable additional burden of disease of  $\leq 10^{-6}$  DALY loss pppy; a more realistic level might be  $\leq 10^{-5}$  DALY loss pppy for consumers of wastewater-irrigated food crops eaten uncooked and  $\leq 10^{-4}$  DALY loss pppy for those who work (or play) in wastewater-irrigated fields. A less stringent level could be set for the latter if they are given the option to make an informed choice regarding their working conditions and thus their occupational health risks (they are a readily identifiable group of people who can be easily given treatment when necessary, for example, oral rehydration salts and anti-helminthic drugs).

Fieldworkers would therefore be protected, at least partially, by wastewater treatment that achieves a pathogen reduction of two orders of magnitude lower than that for  $\leq 10^{-6}$  DALY loss pppy, which is a reduction of only 1–2 log units. Similarly, consumers would be protected by a total pathogen reduction one order of magnitude lower than that for  $\leq 10^{-6}$  DALY loss pppy, which is a reduction of only 1–2 log units by wastewater treatment supplemented by 4–5 log units achieved

by post-treatment health-protection control measures. This is discussed further in this book.

### **SINGLE-EVENT INFECTION RISKS AS A MEASURE OF ‘OUTBREAK POTENTIAL’**

The probability of infection used as a benchmark for acceptability is typically the annualized probability of infection, where independent exposure events throughout the year are used to estimate the annual risk (as presented in the section below). However, the instantaneous level of infection risk to the exposed population fluctuates throughout the year, with disease outbreaks typically associated with shorter-duration periods of heightened risk. Signor and Ashbolt (2009) present a case for the widespread adoption of shorter-duration reference periods (i.e. per exposure or per day) for infection probability targets with which to assess, report and benchmark risks. They argue that doing so may provide opportunities for improved water-related disease risk management, with an incentive to reduce the occurrence and impact of event-driven peaks. Signor and Ashbolt suggest that for a design or operational target of annual disease risk of  $10^{-4}$  per person, a daily or single-exposure disease probability of  $10^{-6}$  per person would meet the aims of the original target, as well as promote the undertaking of measures to control the extent of short-term adverse risk fluctuations. This could be generalized to a single-exposure disease risk of  $10^{-(x+y)}$  pppy for an acceptable annual disease risk of  $10^{-x}$  per person, where the value of  $y$  depends on the frequency of exposure. The corresponding infection risks would, of course, be lower.

### **MORE RIGOROUS METHOD TO ESTIMATE ANNUAL RISKS**

Karavarsamis and Hamilton (2009) recommend a superior method of estimating annual infection risks from QMRA-Monte Carlo simulations. This method is described in detail in Box 5.1 as Approach A. In brief, it appropriately represents daily variation in infection risk in the determination of annual risk, in contrast to the common practice (Approach B) of extrapolating an imprecise estimate of annual risk from infection risk for any one day of exposure (as in the procedure used by Mara et al., 2007, and in the 2006 WHO Guidelines). Karavarsamis and Hamilton point out that repeated calculation through simulation does not solve the shortcomings of the latter approach: it merely generates a distribution of imprecise estimates. Risk estimates resulting from the application of both methods to five wastewater irrigation scenarios, presented in Table 5.1, show that, while the median risks from the two methods are similar, the Karavarsamis and Hamilton method yields 95-percentile risks, which are sometimes used as conservative estimates of annual risk, up to an order of magnitude lower than the WHO (2006) method.

### Box 5.1 IMPROVED REPRESENTATION OF UNCERTAINTY IN ANNUAL INFECTION RISK MODELLING

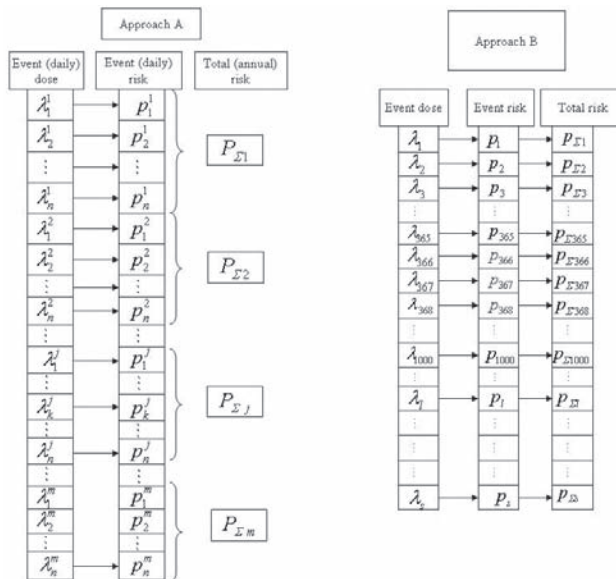
The earliest QMRA methods for wastewater irrigation tended to use straightforward deterministic models, where model parameters are represented by single values (point-estimates) (e.g. Asano et al., 1992; Shuval et al., 1997). More recently, modelling techniques such as Monte Carlo simulation (MCS) have been employed and encouraged in an effort to account for uncertainty (WHO, 2006). However, proper and effective use of these tools involves more than just substituting probability distributions for point-estimates: it demands careful attention to model structure, assumptions and computation.<sup>1</sup>

Having used exposure and dose-response models to determine the infection risk,  $p$ , per exposure event, the total probability of infection over  $n$  exposures,  $P_{\Sigma j}$ , is given as:

$$P_{\Sigma j} = 1 - \prod_{k=1}^n (1 - p_k^j) \quad 5.1$$

where  $p_k^j$  is the infection probability for the  $k^{\text{th}}$  iteration of an exposure event in the  $j^{\text{th}}$  simulation, and where events are assumed to be independent.

Clearly, if one exposure event is assumed to occur each day of the year, then  $p_k^j$  represents a daily risk (i.e.  $n = 365$ ) and  $P_{\Sigma j}$  is an annual risk. MCS can be used to draw realizations of dose,  $\lambda_k^j$ s, from an exposure model, which can then be fed through a dose-response model to yield  $p_k^j$ , and this can be done  $n$  times and Equation 5.1 used to give a single estimate of total risk,  $P_{\Sigma}$  (Figure 5.1). This entire process can then be repeated  $m$  times to obtain a simulated distribution of  $P_{\Sigma j}$ , to obtain a variance of the annual risk estimate. Thus, this approach involves simulations labelled  $j$  ( $j = 1, 2, \dots, m$ ) which comprise iterations labelled  $k$  ( $k = 1, 2, \dots, n$ ).



**Figure 5.1** Schematic of recommended (Approach A) and not recommended (Approach B) methods for determining annual infection risk

If each exposure event is assumed to result in the same (i.e., constant) probability of infection,  $p$ , then Equation 5.1 reduces to:

$$p_{\Sigma} = 1 - (1 - p)^n \quad 5.2$$

for a given simulation,  $i$ . Equation 5.2 is clearly appropriate for a simple deterministic risk assessment, where the infection probability is described by a single constant value,  $p$ , for every exposure event. Often there is only one dose value available and this is then run through a dose-response model to yield a single probability of infection. Equation 5.1 is simply not an option in such circumstances. There are limitations associated with representing dose and consequently infection probability with a single value (Benke and Hamilton, 2008), nevertheless Equation 5.2 is a logical way of determining total risk under the assumption of constant infection probability per exposure event.

However, problems arise when this constant event infection probability assumption is violated. This has mostly occurred in the context of stochastic QMRAs that have used Equation 5.2 with MCS in an attempt to account for uncertainty in the dose distribution (e.g., van Ginneken and Oron, 2000; Hamilton et al., 2006; Mara et al., 2007; Seidu et al., 2008; WHO, 2006). This method is represented schematically in Figure 5.1 as Approach B. For a given simulation,  $i$ , a dose,  $\lambda_i$ , is drawn and, following implementation of the dose-response model, this gives rise to an event infection probability,  $p_i$ . Note that for this approach an iteration is equivalent to a simulation. Next, in an invalid attempt to determine an estimate of total risk, this process is then repeated  $s$  times. The key error in this approach is that the constant event infection probability assumption of Equation 5.1 is not met. Plainly  $p(i = 1, 2, \dots s)$  is not constant for each and every event of  $n$ . Iterating Equation 5.1 thousands of times with a different 'constant' value is simply pseudoreplication as reproducing a component of total risk many times over is not the same as simulating replications of the annual risk itself. The intent of the MCS to characterize uncertainty in total risk estimation is therefore not achieved in Approach B, and consequently Approach A is now recommended.

**Table 5.1** *Comparison of the Karavarsamis and Hamilton (2009) and WHO (2006) methods for determining annual rotavirus infection risks pppy from the consumption of wastewater-irrigated lettuce<sup>a</sup>*

Wastewater quality ( <i>E. coli</i> per 100ml)	Rotavirus infection risk per person per year			
	WHO (2006)		Karavarsamis & Hamilton (2009)	
	Median	95-percentile	Median	95-percentile
$10^7$ – $10^8$	1	1	1	1
$10^3$ – $10^4$	0.29	0.70	0.36	0.39
100–1000	$3.4 \times 10^{-2}$	0.11	$4.5 \times 10^{-2}$	$4.9 \times 10^{-2}$
10–100	$3.5 \times 10^{-3}$	$1.3 \times 10^{-2}$	$4.6 \times 10^{-3}$	$5.1 \times 10^{-3}$
1–10	$3.4 \times 10^{-4}$	$1.2 \times 10^{-3}$	$4.6 \times 10^{-4}$	$5.1 \times 10^{-4}$

<sup>a</sup>Estimated by 10,000 Monte Carlo simulations. Assumptions: 100g lettuce eaten per person per two days; 10–15ml wastewater remaining on 100g lettuce after irrigation; 0.1–1 rotavirus per  $10^5$  *E. coli*; no pathogen die-off;  $N_{50} = 6.7 \pm 25\%$  and  $\alpha = 0.253 \pm 25\%$ .

## ESTIMATES OF NOROVIRUS INFECTION RISKS

The ‘index’ viral pathogen used in the 2006 Guidelines was rotavirus. However, a better index virus is norovirus (NV), which is a very common, if not the commonest, cause of gastroenteritis and certainly the commonest viral cause of gastroenteritis, affecting all age groups (Widdowson et al., 2005) – whereas rotavirus mainly affects children under the age of three – and for which dose-response data are now available (Teunis et al., 2008).

The tolerable NV disease and infection risks corresponding to a tolerable DALY loss of  $10^{-5}$  pppy were determined using a DALY loss of  $9 \times 10^{-4}$  per case of NV disease (Kemmeren et al., 2006) and an NV disease/infection ratio of 0.8 (Moe, 2009) as follows:

$$\text{Tolerable NV disease risk} = \frac{\text{Tolerable DALY loss ppy}}{\text{DALY loss per case of NV disease}} = \frac{10^{-5}}{9 \times 10^{-4}} = 1.1 \times 10^{-2} \text{ pppy} \quad 5.3$$

$$\text{Tolerable NV infection risk} = \frac{\text{Tolerable NV disease risk pppy}}{\text{NV disease/infection ratio}} = \frac{1.1 \times 10^{-2}}{0.8} = 1.4 \times 10^{-2} \text{ pppy} \quad 5.4$$

The NV dose-response dataset of Teunis et al. (2008) was used in place of the beta-Poisson equation in the QMRA-MC computer program developed to determine median NV infection risks pppy (Teunis and Havelaar, 2000); the program was based on the Karavarsamis and Hamilton method described in this section. The resulting estimates of median risk obtained are given in Table 5.2, together with the assumptions on which they are based (which are the same as those used in the 2006 Guidelines but without pathogen die-off) (Mara and Sleight, 2009a). This shows that a reduction of 5 log units results in an NV infection risk of  $2.9 \times 10^{-2}$  pppy, which is only marginally higher than the tolerable NV infection risk of  $1.4 \times 10^{-2}$  pppy determined above.

## ESTIMATES OF *ASCARIS* INFECTION RISKS

The 2006 WHO Guidelines for the safe use of wastewater in agriculture (WHO 2006) make the same recommendation for helminth eggs as was made in the 1989 Guidelines (WHO 1989):  $\leq 1$  human intestinal nematode egg per litre of treated wastewater. The human intestinal nematodes of importance here are *Ascaris lumbricoides* (the human roundworm), *Trichuris trichiura* (the human whipworm), and *Ancylostoma duodenale* and *Necator americanus* (the human hookworms). However, epidemiological studies in Mexico have shown that, while this guideline value protects adults, it does not protect children under the age of 15 (Blumenthal et al., 1996). Blumenthal et al. (2000) therefore recommended lowering the

**Table 5.2** *Median norovirus infection risks per person per year from the consumption of 100g of wastewater-irrigated lettuce every two days<sup>a</sup>*

Wastewater quality ( <i>E. coli</i> per 100ml)	Median norovirus infection risk pppy
10 <sup>7</sup> –10 <sup>8</sup>	1
10 <sup>6</sup> –10 <sup>7</sup>	1
10 <sup>5</sup> –10 <sup>6</sup>	1
10 <sup>4</sup> –10 <sup>5</sup>	0.94
10 <sup>3</sup> –10 <sup>4</sup>	0.25
100–1000	2.9 × 10 <sup>-2</sup>
10–100	2.9 × 10 <sup>-3</sup>
1–10	2.9 × 10 <sup>-4</sup>

<sup>a</sup>Estimated by 10,000 Monte Carlo simulations. Assumptions: 10–15ml wastewater remaining on 100g lettuce after irrigation; 0.1–1 norovirus per 10<sup>5</sup> *E. coli*; no die-off between last irrigation and consumption.

Source: Mara and Sleigh (2009a)

guideline value to ≤0.1 egg per litre wherever children under 15 are exposed and the soil conditions are favourable to egg survival, but this recommendation was not accepted by the international group of experts who participated in the development and review of the Guidelines at a meeting held in Geneva in June 2005, on the grounds that it was too difficult to measure an egg concentration as low as 0.1 per litre. However, if the wastewater is treated in waste stabilization ponds (WSP), which are generally the best wastewater-treatment process in developing countries (Mara, 2004), the egg concentration in the effluent can be simply determined from the egg concentration in the untreated wastewater (which is relatively easy to measure) by using the design equation for egg removal in WSP given by Ayres et al. (1992).

Since the 2006 WHO Guidelines do not protect the health of children under 15 against intestinal nematode disease (unless, additionally, they are dewormed at home or at school), QMRA can be used to determine how best children under 15 can be protected against *Ascaris* infection, now that *Ascaris* dose-response data are available (for details see Chapter 4).

For a tolerable DALY loss of 10<sup>-5</sup> pppy, a DALY loss per case of ascariasis of 8.25 × 10<sup>-3</sup> (Chan, 1997) and, as a worst case scenario, an *Ascaris* disease/infection ratio of 1 (i.e. all those infected with *Ascaris* develop ascariasis), the tolerable *Ascaris* infection risk is given by:

$$\frac{\text{Tolerable DALY loss ppy}}{\text{DALY loss per case of ascariasis}} = \frac{10^{-5}}{8.25 \times 10^{-3}} = 1.2 \times 10^{-3} \text{ pppy} \quad 5.5$$

Median *Ascaris* infection risks pppy from the consumption by children under 15 of raw carrots irrigated with wastewaters containing specified numbers of *Ascaris* eggs were determined by a QMRA-Monte Carlo computer program based on the Karavarsamis and Hamilton method described in this chapter. The resulting estimates of median *Ascaris* infection risk obtained, and the assumptions on which they are based, are given in Table 5.3 (Mara and Sleigh, 2009b). This shows that one egg per litre results in an *Ascaris* infection risk of  $6 \times 10^{-3}$  pppy and 0.1 egg per litre in one of  $6 \times 10^{-4}$  pppy; these risks are higher and lower, respectively, than the tolerable *Ascaris* infection risk of  $10^{-3}$  pppy determined above. This could be taken to confirm the finding of Blumenthal et al. (1996) that  $\leq 1$  egg per litre is not protective of children under 15, and thus reinforce the recommendation of Blumenthal et al. (2000) that, when children under 15 are exposed, the guideline value should be  $\leq 0.1$  egg per litre. However, as noted in the 2006 WHO Guidelines (and in Chapter 3), post-treatment health-protection control measures (Table 5.4) achieve significant pathogen reductions, so that wastewater treatment does not have to achieve the total pathogen reduction required to protect consumer health. This is discussed further below.

### PATHOGEN REDUCTION ACHIEVED BY PRODUCE-WASHING AND DISINFECTION

The 2006 Guidelines allocate a 1 log unit pathogen reduction to washing wastewater-irrigated food crops in clean water, a 2 log unit reduction to produce

**Table 5.3** *Median Ascaris infection risks for children under 15 from the consumption of raw wastewater-irrigated carrots<sup>a</sup>*

Number of <i>Ascaris</i> eggs per litre of wastewater	Median <i>Ascaris</i> infection risk pppy	Notes
100–1000	0.86	Raw wastewaters in hyperendemic areas.
10–100	0.24	Raw wastewaters in endemic areas.
1–10	$2.9 \times 10^{-2}$	Treated wastewaters.
1	$5.5 \times 10^{-3}$	Wastewater quality required to comply with the 1989 and 2006 WHO Guidelines.
0.1–1	$3.0 \times 10^{-3}$	Highly treated wastewaters.
0.1	$5.5 \times 10^{-4}$	Wastewater quality recommended by Blumenthal et al. (2000).
0.01–0.1	$3.0 \times 10^{-4}$	Treated wastewaters in non-endemic areas.

<sup>a</sup>Estimated by 10,000 Monte Carlo simulations. Assumptions: 30–50g raw carrots consumed per child per week (Navarro et al., 2009); 3–5ml wastewater remaining on 100g carrots after irrigation (Mara et al., 2007);  $N_{50} = 859 \pm 25\%$  and  $\alpha = 0.104 \pm 25\%$ ; no *Ascaris* die-off between final irrigation and consumption.  
Source: Mara and Sleigh (2009b)



disinfection and also a 2 log unit reduction to produce peeling. Amoah et al. (2007) investigated 'common and improved sanitary washing methods for the reduction of coliform bacteria and helminth eggs on vegetables' in urban West Africa, where 56–90 per cent of the households and 80–100 per cent of the restaurants were found to use some kind of disinfectant for washing leafy vegetables to be eaten raw, with the rest using only water. In laboratory studies produce disinfection with Eau de Javel® (a chlorine solution commonly used for salad washing in francophone West Africa) achieved a 3-log unit reduction of faecal coliforms on lettuce after a contact time of ten minutes and subsequent rinsing in clean water. Helminth eggs were most effectively removed from lettuce by washing with water under an open tap; this achieved a reduction from nine eggs per 100g to one egg per 100g. More details on this are in Chapter 12.

### **APPLICATION TO URBAN AGRICULTURE IN DEVELOPING COUNTRIES**

Exposure varies due to differences in consumption patterns which need to be accounted for in the risk calculations. For example, Seidu et al. (2008) reported that people in urban Ghana commonly consume 10–12g of lettuce in 'fast food' on each of four days per week. This refers to a specific situation in one developing country and this may or may not be representative of what happens elsewhere, but it is much less than the 100g of lettuce consumed on alternate days used by Shuval et al. (1997) to reflect the situation in Israel. Infection risks for this Ghanaian consumption of lettuce were simulated by a QMRA-Monte Carlo computer program based on the Karavarsamis and Hamilton method described in this chapter. The resulting risks, together with the assumptions on which they are based, are given in Table 5.4, which shows that a reduction of 4 log units results in a norovirus infection risk of  $3.6 \times 10^{-2}$  pppy, which is only marginally higher than the tolerable norovirus infection risk determined in the section for a tolerable DALY loss of  $10^{-5}$  pppy. (Of course, if a larger quantity of lettuce were to be consumed, then the risk of infection would be correspondingly higher.) The required 4 log unit reduction (Table 5.4) could be achieved by, for example, a 1 log unit reduction by wastewater treatment and a 3 log unit reduction by produce disinfection (or, if disinfection is not routinely or reliably practised, a 2 log unit reduction through die-off and a 1 log unit reduction by produce-washing in clean water).

#### **Implications for wastewater treatment**

In the above example wastewater treatment is required to produce only a single log unit pathogen reduction. This can be readily achieved by very simple treatment processes, such as an anaerobic pond, a three-tank or three-pond system and

**Table 5.4** *Median norovirus infection risks pppy from the consumption of 10–12g of wastewater-irrigated lettuce on four occasions per week<sup>a</sup>*

Wastewater quality ( <i>E. coli</i> per 100ml)	Median norovirus infection risk pppy
$10^7$ – $10^8$	1
$10^6$ – $10^7$	1
$10^5$ – $10^6$	0.97
$10^4$ – $10^5$	0.30
$10^3$ – $10^4$	$3.6 \times 10^{-2}$
100–1000	$3.6 \times 10^{-3}$
10–100	$3.6 \times 10^{-4}$
1–10	$3.6 \times 10^{-5}$

<sup>a</sup>Estimated by 10,000 Monte Carlo simulations. Assumptions: 10–15ml wastewater remaining on 100g lettuce after irrigation; 0.1–1 norovirus per  $10^5$  *E. coli*; no die-off between last irrigation and consumption.

overnight settling. The three-tank or three-pond system is operated as a sequential batch-fed process: on any one day one tank or pond is filled with wastewater, the contents of another are settling and the contents of the third are used for irrigation. This is a very reliable, almost foolproof system. In small-scale urban agriculture, as opposed to large-farm agriculture, a single tank is generally sufficient (and more affordable): on any day in the morning the tank contents are used for crop watering, and the tank is then refilled and its contents allowed to settle until the following morning.

For helminth eggs, if it is assumed that in areas where ascariasis is endemic untreated wastewater contains 100 *Ascaris* eggs per litre, a 3 log unit egg reduction is required to achieve 0.1 egg per litre. For root vegetables eaten raw and assuming that a 2 log unit reduction occurs through produce peeling prior to consumption (WHO, 2006), wastewater treatment is required to effect a reduction of 1 log unit from 100 to 10 eggs per litre. This reduction can also be achieved by any of the three methods described above. In hyperendemic areas (1000 eggs per litre of untreated wastewater) a further log unit reduction is required; this could be achieved by rinsing the peeled produce in a weak detergent solution and rinsing with clean water.

## NOTE

- 1 The QMRA-Monte Carlo computer programs used in the preparation of this chapter are available at [www.personal.leeds.ac.uk/~cen6ddm/QMRA.html](http://www.personal.leeds.ac.uk/~cen6ddm/QMRA.html). All these programs, with the exception of the one for *Ascaris*, use a range of pathogen-to-*E. coli* numbers – for example, 0.1–1 pathogen per  $10^5$  *E. coli*. This approach was taken by Shuval et al. (1997) and adopted in the 2006 WHO Guidelines, as there are very few, and in many situations no, data on pathogen numbers in developing-country wastewaters,

whereas *E. coli* numbers are available or, if not available, are easy to obtain. However, setting the range of pathogen numbers to  $10^5$ – $10^5$  per  $10^5$  *E. coli* in the QMRA-MC programs (i.e., equating pathogen and *E. coli* numbers) means that the programs determine the pathogen risks directly, so that the first column in Tables 5.1, 5.2 and 5.4 would express the wastewater quality in terms of a range of pathogen numbers per 100ml (or any other desired unit volume), rather than as a range of *E. coli* numbers per 100ml.

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