Annex A: Development of Guidelines for Wastewater Use in Agriculture 1918-2010

Part A1: Standards and Guidelines 1918−1989

A1.1 State of California

The Californian 'Title 22' regulations for the quality of treated wastewater to be used for crop irrigation were first promulgated in 1918 (Asano and Levine 1996). Currently the regulations are (State of California 2001):

- for restricted irrigation: ≤23 total coliforms per 100 mL, and
- for unrestricted irrigation: ≤2.2 total coliforms per 100 mL.

These regulations are very strict and are based on potential, rather than actual, risks to health − i.e., they are not based on any epidemiological evidence, but only on the possibility that pathogens *may* be present and that therefore there *may* be a risk to human health (see Box A1.1).

Box A1.1 Actual and potential health risks in wastewater irrigation

An ***actual*** risk to public health occurs as a result of wastewater irrigation when <u>all</u> of the following four conditions are satisfied:

(1) *either* an infective dose of the pathogen reaches the wastewater-irrigated field *or* the pathogen multiples in the field to form an infective dose,
(2) the infective dose reaches a human host,
(3) the host becomes infected, <u>and</u>
(4) the infection causes disease or further transmission.

Actual risks can thus only be determined from epidemiological studies.

If conditions 1−3 are satisfied but <u>not</u> condition 4, then the risk is only a ***potential*** risk.

Source: WHO 1989.
Note: this Box is the same as Box 3.2 and is reproduced here for ease of reference.

A1.2 United States of America

The US Environmental Protection Agency recommended that river waters used for unrestricted irrigation should contain ≤1000 fecal coliforms per 100 mL (USEPA 1973). However, the US EPA and the United States Agency for International Development have jointly published much stricter guidelines[35] for wastewater use in agriculture (USEPA/USAID 1992, 2004), as follows:

- for the irrigation of "food crops not commercially processed − surface or spray irrigation of any food crop, including crops eaten raw" (i.e., unrestricted irrigation): no detectable fecal coliforms per 100 mL; and

- for the irrigation of "food crops commercially processed − surface irrigation of orchards and vineyards", and "non-food crops − pasture for milking animals, fodder, fiber, and seed crops" (i.e., restricted irrigation): ≤200 fecal coliforms per 100 mL.

The guideline for unrestricted irrigation is stricter than the corresponding Californian standard (and, it may be noted, is the same as that required for drinking water), but that for restricted irrigation is less strict by an order of magnitude. The rationale for these guidelines was not given, but it is clear that they were based on only potential health risks.

A1.3 World Health Organization

The World Health Organization has published three editions of its Guidelines for the safe use of wastewater in agriculture: in 1973, 1989, and 2006. The salient points of the 1973 and 1989 editions are discussed below [the 2006 edition is discussed in Part 2 of this Annex].

►*The 1973 WHO Guidelines*

The 1973 Guidelines (WHO 1973) "recognized that the extremely strict Californian standards were not justified by the available epidemiological evidence", and made the following recommendations:

- for both restricted and unrestricted irrigation: "no chemicals which lead to undesirable residues in crops or fish",

- additionally for unrestricted irrigation ("crops eaten raw"): ≤100 total coliforms per 100 mL, and

- additionally for restricted irrigation − (a) for "crops not for direct human consumption": "freedom from gross solids, [and] significant removal of parasite eggs"; and (b) *either* ≤100 total coliforms per 100 mL *or* "significant removal of bacteria".

---

[35] In the USA it is the states that have the jurisdiction to establish legally enforceable standards. Federal agencies (like US EPA and USAID) can only make recommendations in the form of guidelines.

The 'undesirable residues' and 'significant removals' were not defined and the Guidelines were simply interpreted as requiring ≤100 total coliforms per 100 mL for unrestricted irrigation.

The 1989 WHO Guidelines

The recommendations in the 1989 Guidelines (WHO 1989), based largely on the epidemiological evidence reviewed by Shuval et al. (1986) and the recommendations made in the *Engelberg Report* (Shuval and Mara 1985), are as follows:

- *Restricted irrigation*: ≤1 human intestinal nematode egg per liter; and
- *Unrestricted irrigation:* ≤1 human intestinal nematode egg per liter and ≤1000 fecal coliforms per 100 mL.

The 1989 Guidelines incorporated the distinction between actual and potential risks (Box A1.1) and introduced 'health-protection control measures' (crop restriction, drip irrigation and human exposure control) which could be used, either singly or in combination, when wastewater treatment does not, or is unable to, achieve ≤1 egg per liter and ≤1000 fecal coliforms per 100 mL (Figures A1.1 and A1.2).

The 1989 Guidelines were not without their critics. Some considered that the fecal coliform guideline value of ≤1000 per 100 ml far too lax (for example: Shelef 1991) or that it was too difficult to measure a helminth egg concentration as low as 1 per liter (but see Part 4 of this Annex). However, most of the criticism was centered on the requirement for wastewater treatment to achieve ≤1000 fecal coliforms per 100 mL, especially in low- and middle-income countries where little wastewater is effectively treated or not treated.

This dissatisfaction with the apparent lack of applicability of the 1989 WHO Guidelines in the 'real world', where most wastewater used for large-farm irrigation and in urban agriculture is untreated, gave rise to the 'Hyderabad Declaration' (IWMI 2002), which called for the "development and application of guidelines for **untreated** wastewater use that safeguard livelihoods, public health and the environment" [emphasis added]. This, in turn, has led to work on 'non-treatment options' (*cf.* section 4.3.2).
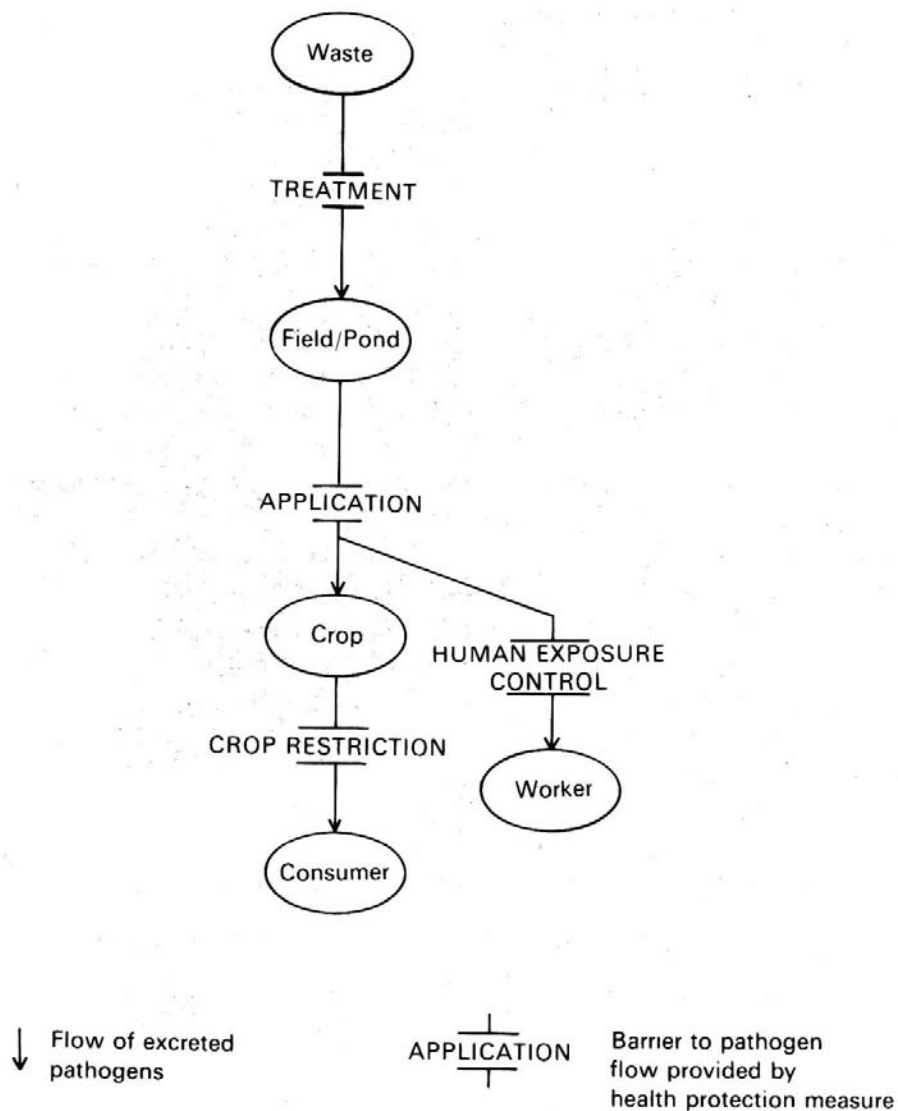
Figure A1.1. Effect of health-protection control measures in interrupting transmission routes of excreted pathogens in wastewater use in agriculture

'Treatment' is either treatment to ≤1 helminth egg per liter and ≤1000 fecal coliforms per 100 mL or partial treatment to a lower quality. 'Application' refers specifically to the use of drip irrigation, 'Crop restriction' to not irrigating salad crops and vegetables that may be eaten uncooked, and 'Human exposure control' to the provision of protective clothing, gloves, footwear, and handwashing facilities to fieldworkers.

Note: the four 'barriers to pathogen flow' can be used as 'HACCP critical control points' (section A4.2).

Sources: Blumenthal 1988, Blumenthal et al. 1989, and WHO 1989.
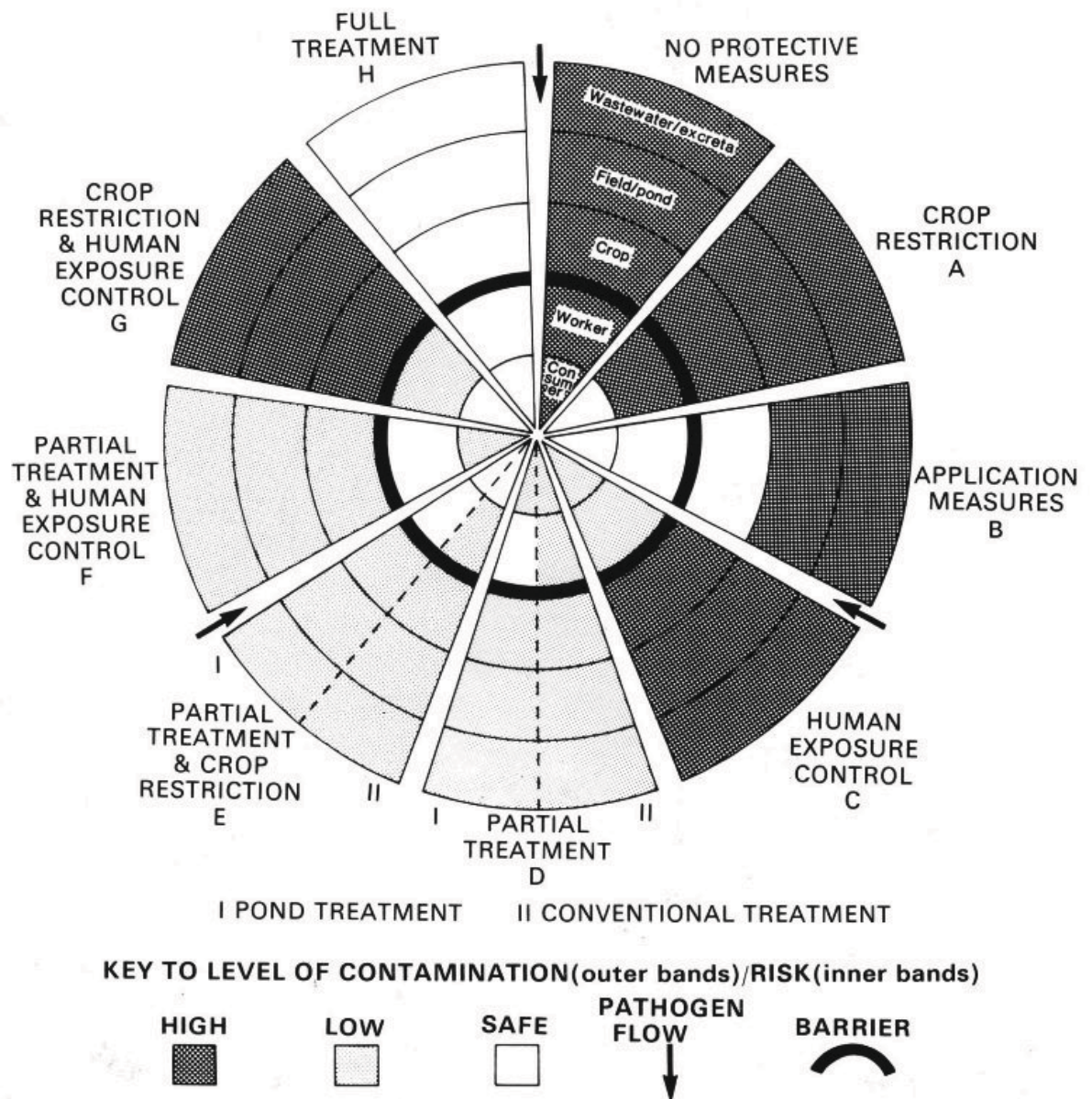
109

Figure A1.2. Generalized model showing the level of risk to human health associated with different combinations of health-protection control measures for wastewater use in agriculture.

'Full treatment' is treatment to ≤1 helminth egg per liter and ≤1000 fecal coliforms per 100 mL. 'Crop restriction' refers to not irrigating salad crops and vegetables that may be eaten uncooked, 'Application measures' to the use of drip irrigation, and 'Human exposure control' to the provision of protective clothing, gloves, footwear, and handwashing facilities, to fieldworkers.

Sources: Blumenthal 1988, Blumenthal et al. 1989, and WHO 1989.

Annex A–Part 2
Quantitative Microbial Risk Analysis: The 2006 WHO Guidelines

A2.1 Introduction to the Basic Concepts of QMRA

QMRA: what it can do

Quantitative microbial risk analysis (QMRA) determines a numerical value of the risk (i.e., probability) of disease and/or infection as a result of a person or a community being exposed to a specified number of a particular pathogen (referred to in the equations below as the pathogen dose *d*) as a result of some activity – here the relevant activities are consuming wastewater-irrigated foods and working in wastewater-irrigated fields. QMRA can be used to estimate disease and infection risks for any pathogen as long as there are dose-response data available for it; the disease and infection risks can then be estimated by using one of the following two QMRA dose-response equations (Haas et al. 1999):

   (a) Exponential dose-response equation (commonly used for protozoan pathogens):

$$P_I(d) = 1 - e^{-rd} \qquad (A2.1)$$

   (b) Beta-Poisson dose-response equation (commonly used for viral and bacterial pathogens):

$$P_I(d) = 1 - \left(1 + \frac{d}{N_{50}}\left(2^{1/\alpha} - 1\right)\right)^{-\alpha} \qquad (A2.2)$$

where $P_I(d)$ is the risk of infection in an individual from a single exposure to (here, the ingestion of) a single pathogen dose *d*; N_{50} is the median infective dose (i.e., the value of *d* that causes infection in 50% of the exposed population); and α and *r* are pathogen 'infectivity constants'.

The annual risk of infection is given by:

$$P_{I(A)}(d) = 1 - [1 - P_I(d)]^n \qquad (A2.3)$$

where $P_{I(A)}(d)$ is the annual risk of infection in an individual from *n* exposures per year to the single pathogen dose d − the derivation of equation A2.3 is given in Box A2.1.

The risk of disease, as opposed to the risk of infection, is given by:

$$P_D(d) = \delta P_I(d) \qquad (A2.4)$$

111

where $P_D(d)$ is the risk of disease in an individual from a single exposure to the single pathogen dose d; and δ is the disease/infection ratio (i.e., the proportion of the infected population that becomes clinically ill; thus the value of δ is in the range 0−1).

The use of equations A2.2 and A2.3 is illustrated in Box A2.2.

Box A2.1: Derivation of Equation A2.3

As noted above, $\mathbf{P_I}(\boldsymbol{d})$ is the risk (i.e., probability) of infection per person per single exposure to a pathogen dose d.

► Therefore the risk of a person *not* becoming infected from a single exposure to this pathogen dose d is $[\mathbf{1 - P_I}(\boldsymbol{d})]$, as the risk of becoming infected and the risk of not becoming infected must add up to 1.

► Therefore the risk of a person *not* becoming infected following n exposures per year to the pathogen dose d is $[\mathbf{1 - P_I}(\boldsymbol{d})]^{\boldsymbol{n}}$.

► Therefore the risk of a person *becoming* infected following n exposures per year to the patho-gen dose d [this is $P_{I(A)}(d)$] is $\mathbf{1 - [1 - P_I}(\boldsymbol{d})]^{\boldsymbol{n}}$.

Box A2.2: Example of a QMRA risk determination

Suppose that:

(a) the pathogen dose d is 1×10^{-5} – this means that, if this dose is contained in 10 g of lettuce (which could be consumed in, for example, a fast-food sandwich or burger), the pathogen concentration is 1 per 10,000 g (i.e., 1 per 10 kg) of lettuce, which is a very low concentration, but one that nevertheless poses a health risk.

(b) the pathogen is *Campylobacter* (the commonest bacterial cause of diarrhea worldwide), for which $N_{50} = 896$ and $\alpha = 0.145$. Then, from equation A2.2:

$$P_I(d) = 1 - [1 + (1 \times 10^{-5}/896)(2^{1/0.145} - 1)]^{-0.145} = 2 \times 10^{-7}$$

(c) an individual eats a sandwich containing 10 g of lettuce containing 1 *Campylobacter* per 10 kg every working day of the year (i.e., on 5×50 days per year). Then, from equation A2.3:

$$P_{I(A)}(d) = 1 - [1 - (2 \times 10^{-7})]^{(5 \times 50)} = 5 \times 10^{-5}$$

Thus the individual has a 5 in 100,000 (i.e., a 1 in 20,000) chance of being infected with *Campylobacter* in any 12-month period – or, put another way, if in a large city a million people eat these sandwiches every working day of the year, then 50 people will become infected with *Campylobacter* each year.

Interpreting values of risk

Values of risk (probability) are in the range 0−1 and are expressed per person per exposure event or, more commonly in relation to wastewater use in agriculture, per person per year (pppy). For example, an infection risk of 0.01 (i.e., 1×10^{-2}) pppy means that each year an exposed individual has a 1% chance of becoming infected as a result of n exposures per year. Alternative ways of interpreting this infection risk of 0.01 pppy are (a) that the exposed individual will become infected once every 100 years (i.e., essentially once in his or her lifetime), or (b) for a community an infection risk of 0.01 pppy means that every year 1% of the community will become infected.

Note: in practice there is no such thing as a 'zero risk'. A risk can be very small—for example, 1×10^{-x}, where x is very large—but it is <u>not</u> zero.

Monte Carlo risk simulations

In the example QMRA risk determination in Box A2.2 'fixed' values of the parameters are used – for example, N_{50} and α are taken to be *exactly* 896 and 0.145, respectively. However, these values are subject to some uncertainty and it is possible that the value of N_{50}, for example, could be anywhere in the range 800−1000 or even 700−1100. To overcome, at least partially, the uncertainty of many of the parameters used in QMRA risk determinations, Monte Carlo risk simulations are used in which a *range of values* is assigned to each parameter used in the calculations, rather than a *single fixed value*, although a fixed value can be assigned to any given parameter if so desired. A computer program then selects at random a value for each parameter from the range of values specified for it and then determines the resulting annual risk. The program then repeats this process a large number of times (generally for a total of 1000 or 10,000 times) and determines the median and 95-percentile risks. This large number of repetitions removes some of the uncertainty associated with the parameter values and makes the results generated by multi-simulation QMRA-Monte Carlo risk analyses much more robust than those determined by simple QMRA calculations of the type shown in Box A2.2 (and also in Box A2.3), although of course they are only as good as the assumptions made. Section A2.3 illustrates the use of this 'QMRA-MC' approach to risk determination.

A2.2   Tolerable Maximum Additional Burden of Disease and Resulting Tolerable Maximum Disease and Infection Risks

A tolerable maximum additional burden of disease resulting from exposure to a particular activity (for example, the consumption of wastewater-irrigated food) has to be selected. Burdens of disease are now expressed as 'DALY losses' – disability-adjusted life years lost (see Box 4.1 for a description of DALYs) – per person per year (pppy). The DALY loss associated with a case of a disease can be considered as the 'health cost' of one episode of that disease.

Tolerable DALY losses are small – for example, 1×10^{-n} pppy, where *n* is in the range 1−10 (in order of magnitude terms). Once the tolerable additional burden of disease resulting from exposure to a specified activity has been selected, then this tolerable DALY loss pppy has to be 'translated' into tolerable disease and infection risks pppy, as follows:

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

$$\text{Tolerable infection risk pppy} = \frac{\text{Tolerable disease risk pppy}}{\text{Disease/infection ratio}}$$

A2.3 QMRA in the 2006 WHO Guidelines

Tolerable maximum DALY loss

The tolerable maximum additional burden of disease resulting from working in wastewater-irrigated fields and consuming wastewater-irrigated food used in the 2006 WHO Guidelines

is 1×10^{-6} pppy (WHO, 2006), which is the same as that used in the third edition of the WHO Guidelines on Drinking-water Quality (WHO 2004, 2008a). In the drinking-water Guidelines a 10^{-6} DALY loss pppy was chosen as it corresponds to a lifetime excess risk of $\sim 10^{-5}$ per person of dying from a fatal cancer induced by drinking fully treated drinking water containing the maximum permitted concentration of the carcinogen inducing the cancer − this tolerable fatal cancer risk was used by WHO in the second edition of its Guidelines on drinking-water quality (WHO, 1996) and is based on US EPA's acceptance of a 10^{-5}−10^{-6} fatal lifetime waterborne cancer risk (Munro and Travis 1986) (see section A3.1 for further discussion on this point).

The use of the same tolerable maximum additional burden of disease in both the Drinking-water Guidelines and the Wastewater-use Guidelines reflects the reasoning that all water-related diseases, whether waterborne or resulting from wastewater irrigation, should present the same risk to health − this is the basis of the Stockholm Framework (Fewtrell and Bartram 2001) − as people expect the food they eat to be as safe as the water they drink.

Reference pathogens

The 2006 WHO Guidelines considered the following three 'reference' pathogens:
- rotavirus (a viral pathogen),
- *Campylobacter* (a bacterial pathogen), and
- *Cryptosporidium* (a protozoan pathogen).

These pathogens were chosen as 'reference' pathogens because (1) dose-response data were available for them; and (2) they are all epidemiologically important agents of severe diarrhea.

They have the following values for the infectivity constants in equations A2.1 and A2.2:

- rotavirus: $N_{50} = 6.17$ and $\alpha = 0.253$

- *Campylobacter*: $N_{50} = 896$ and $\alpha = 0.145$

- *Cryptosporidium*: $r = 0.0042$

Resulting tolerable disease and infection risks

Tolerable disease and infection risks for rotavirus, *Campylobacter* and *Cryptosporidium* were calculated on the basis of a tolerable maximum DALY loss of 10^{-6} pppy, using the descriptive equations in section A2.2, as shown in Table A2.1, from which a general 'design' value of 10^{-3} pppy was chosen for the tolerable risk of rotavirus infection.

Table A2.1. DALY losses, tolerable disease risks, disease/infection ratios and tolerable infection risks for rotavirus, *Campylobacter* and *Cryptosporidium*

Pathogen	DALY loss per case of disease	Tolerable disease risk pppy for 10^{-6} DALY loss pppy	Disease/ infection ratio	Tolerable infection risk pppy
Rotavirus: (1) IC[a]	1.4×10^{-2}	7.1×10^{-5}	0.05	1.4×10^{-3}
(2) DC[a]	2.6×10^{-2}	3.8×10^{-5}	0.05	7.7×10^{-4}
Campylobacter	4.6×10^{-3}	2.2×10^{-4}	0.7	3.1×10^{-4}
Cryptosporidium	1.5×10^{-3}	6.7×10^{-4}	0.3	2.2×10^{-3}

[a] IC, industrialized countries; DC, developing countries. The DALY loss per case of rotavirus diarrhea is higher in DC than IC because a higher proportion of children under 5 in DC become infected, the disease has a longer duration due to their lower nutritional status, and they form a higher percentage of the total population.

Source: WHO 2006b.

QMRA applied to unrestricted irrigation

QMRA risk estimates are used in the 2006 WHO Guidelines to determine the required reduction of the viral, bacterial and protozoan reference pathogens such that the maximum tolerable DALY loss of 10^{-6} pppy is not exceeded – i.e., for a tolerable rotavirus infection risk of 10^{-3} pppy. The procedure (using fixed parameter values) is illustrated in Box A2.3 for the consumption of wastewater-irrigated lettuce. The calculations show that, for the parameter values selected, the required rotavirus reduction from raw wastewater to lettuce ingestion is 6 log units.[36] This total reduction is achieved partially by wastewater treatment and partially by a selection of the post- treatment (but pre-ingestion) health-protection control measures detailed in Table A2.2.

---

[36] These are $\log_{10}$ units. A 6-log unit reduction is thus a percentage reduction of 99.9999%.

Box A2.3: Specimen QMRA calculation

These specimen calculations illustrate how QMRA can be used to determine the pathogen reduction required to protect consumer health in the case of unrestricted irrigation. Here, as an example, rotavirus infection risks are determined for the exposure scenario of consuming wastewater-irrigated lettuce.

1. Tolerable risk of infection: the 'design' risk of rotavirus infection is taken as 10^{-3} pppy.

2. QMRA equations: equation A2.1 gives the infection risk per person from ingesting a dose (i.e., number) of d pathogens (here, rotavirus) on one occasion [$P_I(d)$] − this is the beta-Poisson equation which is used for viral pathogens:

$$P_I(d) = 1 - [1 + (d/N_{50})(2^{1/\alpha} - 1)]^{-\alpha} \tag{A2.1}$$

where N_{50} is the median infective dose and α is a pathogen 'infectivity constant' (for rotavirus $N_{50} = 6.2$ and $\alpha = 0.253$).

Equation A2.3 gives the annual risk of infection per person [$P_{I(A)}(d)$] resulting from n exposures per year to the pathogen dose d:

$$P_{I(A)}(d) = 1 - [1 - P_I(d)]^n \tag{A2.3}$$

Consumer exposure to rotavirus infection is calculated by using the following illustrative parameter values:

    5000 rotaviruses per liter of untreated wastewater,

    10 mL of treated wastewater remaining on 100 g lettuce after irrigation, and

    100 g lettuce consumed per person every two days throughout the year.

The rotavirus dose per exposure (d) is the number of rotaviruses on 100 g lettuce at the time of consumption. This dose is determined by QMRA as follows:

(a) Conversion of the tolerable rotavirus infection risk of 10^{-3} pppy [$P_{I(A)}(d)$ in equation A2.3] to the risk of infection per person per exposure event [$P_I(d)$ in equation A2.1] − i.e., per consumption of 100 g lettuce, which takes place every two days throughout the year, so n in equation A2.3 is 365/2:

$$P_I(d) = 1 - (1 - 10^{-3})^{[1/(365/2)]} = 5.5 \times 10^{-6}$$

(b) Calculation of the dose per exposure event from a rearrangement of equation A2.1:

$$d = \{[1 - P_I(d)]^{-1/\alpha} - 1\} / \{N_{50}/(2^{1/\alpha} - 1)\}$$

Thus:

$$d = \{[1 - (5.5 \times 10^{-6})]^{-1/0.253} - 1\} / \{6.17/(2^{1/0.253} - 1)\} = 5 \times 10^{-5} \text{ per exposure event}$$

3. Required pathogen reduction: this dose d of 5×10^{-5} rotavirus is contained in the 10 mL of treated wastewater remaining on the lettuce at the time of consumption, so the rotavirus concentration is 5×10^{-5} per 10 mL – i.e., 5×10^{-3} per liter. The number of rotaviruses in the raw wastewater is 5000 per liter and therefore the required total rotavirus reduction in log units is:

$$\log(5000) - \log(5 \times 10^{-3}) = 3.7 - (-2.3) = \mathbf{6}$$

Table A2.2. Health-protection control measures and associated pathogen reductions

Control measure	Pathogen reduction (log units)	Notes
A. Wastewater treatment	1−7	Pathogen reduction depends on type and degree of treatment selected.
B. On-farm options		
Crop restriction (i.e., no food crops eaten uncooked)	6−7	Depends on (a) effectiveness of local enforcement of crop restriction, and (b) comparative profit margin of the alternative crop(s).
On-farm treatment:		
(a) Three-tank system	1−2	System described in section 4.3.1.
(b) Simple sedimentation	0.5−1	Sedimentation for ~18 hours (section 4.3.1).
(c) Simple filtration	1−3	Value depends on filtration system used (section 4.3.2).
Method of wastewater application:		
(a) Furrow irrigation	1−2	Crop density and yield may be reduced.
(b) Low-cost drip irrigation	2−4	2-log unit reduction for low-growing crops, and 4-log unit reduction for high-growing crops.
(c) Reduction of splashing	1−2	Farmers trained to reduce splashing when watering cans used (splashing adds contaminated soil particles on to crop surfaces which can be minimized).
Pathogen die-off	0.5−2 per day	Die-off between last irrigation and harvest (value depends on climate, crop type, etc.).
C. Post-harvest options at local markets		
Overnight storage in baskets	0.5−1	Selling produce after overnight storage in baskets (rather than overnight storage in sacks or selling fresh produce without overnight storage).
Produce preparation prior to sale	1−2	(a) Rinsing salad crops, vegetables and fruit with clean water.
	2−3	(b) Washing salad crops, vegetables and fruit with running tap water.
	1−3	(c) Removing the outer leaves on cabbages, lettuces, etc.
D. In-kitchen produce-preparation options		
Produce disinfection	2−3	Washing salad crops, vegetables and fruit with an appropriate disinfectant solution and rinsing with clean water.
Produce peeling	2	Fruits, root crops.
Produce cooking	5−6	Option depends on local diet and preference for cooked food.

Sources: EPHC/NRMMC/AHMC 2006, WHO 2006b, Amoah et al. 2007b, Abaidoo et al. 2010, and Keraita et al. 2010. *Note:* this table is the same as Table 4.2; it is repoduced here for ease of reference.

Table A2.3 shows the QMRA-MC risk simulations given in the Guidelines for the consumption of wastewater-irrigated lettuce − but with one important difference: no pathogen die-off is assumed, so that the required *total* pathogen reduction is determined (i.e., from raw wastewater to ingestion). This was done since die-off is a *post*-treatment health-protection control measure which is more usefully determined separately, rather than being part of the risk analysis (as was done in the 2006 WHO Guidelines), so that an appropriate local selection of treatment and post-treatment control measures can be consciously made from Table A2.2.

In these risk simulations, as in those in the Guidelines, wastewater quality is expressed as a single-log range of *E. coli* per 100 mL − i.e., 10^x-10^{x+1} [thus 10^7-10^8 per 100 mL represents raw wastewater, and 1−10 per 100 mL represents 'Californian' treated wastewater – i.e., ≤2.2 total coliforms per 100 mL (section A1.1)]. The risk simulations also incorporate a range of pathogen numbers per 10^5 *E. coli* (see section 4.2; *cf.* Shuval et al. 1997).

Table A2.3 shows that the tolerable rotavirus infection risk of 10^{-3} pppy is achieved when the wastewater quality has been reduced from 10^7-10^8 per 100 mL to 1−10 per 100 mL—i.e., a total pathogen reduction of 6 log units. This could be achieved, for example, by wastewater treatment (4 log units – as required for restricted irrigation, see below) and pathogen die-off (2 log units) (*cf.* Table A2.2). In Table A2.3 it is noteworthy that in all cases the *Campylobacter* and *Cryptosporidium* infection risks are lower than those for rotavirus.

The Guidelines also include a 'reverse' QMRA-MC method to determine the log unit pathogen reduction required for a given rotavirus infection risk (rather than determining the infection risk for a given wastewater quality), as shown in Table A2.4.

Table A2.3. Unrestricted irrigation: median infection risks from the consumption of wastewater-irrigated lettuce estimated by 10,000 Monte Carlo simulations[a]

Wastewater quality (*E. coli* per 100 mL)	Infection risk per person per year		
	Rotavirus	*Campylobacter*	*Cryptosporidium*
$10^7 - 10^8$	1	1	0.91
...			
$10^3 - 10^4$	0.30	1.1×10^{-2}	2.4×10^{-4}
100−1000	3.4×10^{-2}	1.1×10^{-3}	2.3×10^{-5}
10−100	3.5×10^{-3}	1.1×10^{-4}	2.3×10^{-6}
1−10	3.5×10^{-4}	1.1×10^{-5}	2.3×10^{-7}

[a]Assumptions: 100 g lettuce eaten per person per 2 days; 10−15 mL wastewater remaining on 100 g lettuce after irrigation; 0.1−1 rotavirus and *Campylobacter*, and 0.01−0.1 *Cryptosporidium* oocyst, per 10^5 *E. coli*; no pathogen die-off; $N_{50} = 6.7 \pm 25\%$ and $\alpha = 0.253 \pm 25\%$ for rotavirus; $N_{50} = 896 \pm 25\%$ and $\alpha = 0.145 \pm 25\%$ for *Campylobacter*; $r = 0.0042 \pm 25\%$ for *Cryptosporidium*.

Source: adapted from WHO 2006b.

Table A2.4. Unrestricted irrigation: required rotavirus reductions for various levels of tolerable risk of rotavirus infection from the consumption of wastewater-irrigated lettuce and onions estimated by 10,000 Monte Carlo simulations[a]

Tolerable level of rotavirus infection risk (pppy)	Corresponding required level of rotavirus reduction (log units)	
	Lettuce	Onions
10^{-1}	4	5
10^{-2}	5	6
10^{-3}	6	7

[a]Assumptions: 100 g lettuce and onions eaten per person per 2 days; 10−15 mL and 1−5 mL wastewater remaining after irrigation on 100 g lettuce and 100 g onions, respectively; 0.1−1 rotavirus per 10^5 *E. coli*; $N_{50} = 6.17 \pm 25\%$ and $\alpha = 0.253 \pm 25\%$; no pathogen die-off.

Source: WHO 2006b.

QMRA applied to restricted irrigation

The exposure scenario for restricted irrigation used in the 2006 WHO Guidelines is the involuntary ingestion of soil particles by those working in wastewater-irrigated fields. This is a likely scenario as wastewater-saturated soil would contaminate the workers' fingers and some pathogens could be transmitted to their mouths and hence ingested. The quantity of soil involuntarily ingested in this way has been reported (but not specifically for this restricted-irrigation scenario) as up to 100 mg per person per day of exposure (Haas et al. 1999, WHO 2001). Two 'sub-scenarios' were investigated: (a) highly mechanized agriculture and (b) labor-intensive agriculture – the former to represent exposure in industrialized countries where farm workers typically plough, sow and harvest using tractors and associated equipment and could be expected to wear gloves when working in wastewater-irrigated fields; and the latter to be representative of farming practices in developing countries in situations where tractors are not (or only rarely) used and gloves not worn. Table A2.5 gives the QMRA-MC risk determinations for labor-intensive agriculture, from which it can be seen that the tolerable rotavirus infection risk of 10^{-3} pppy is achieved by a 4-log unit pathogen reduction (from 10^7–10^8 *E. coli* per 100 g of soil to 10^3–10^4 per 100 g). This pathogen reduction has to be achieved by treatment as there is no other way of protecting the fieldworkers (and therefore this level of treatment, supplemented by some of the post-treatment health protection control measures detailed in Table A2.2, is also used for unrestricted irrigation).

Table A2.5. Restricted irrigation: labor-intensive agriculture with exposure for 300 days per year − median infection risks from involuntary ingestion of wastewater-contaminated soil estimated by 10,000 Monte Carlo simulations[a]

Soil quality (*E. coli* per 100 g)[b]	Infection risk per person per year		
	Rotavirus	*Campylobacter*	*Cryptosporidium*
10^7-10^8	0.99	0.50	1.4×10^{-2}
10^6-10^7	0.88	6.7×10^{-2}	1.4×10^{-3}
10^5-10^6	0.19	7.3×10^{-3}	1.4×10^{-4}
10^4-10^5	2.0×10^{-2}	7.0×10^{-4}	1.3×10^{-5}
10^3-10^4	1.8×10^{-3}	6.1×10^{-5}	1.4×10^{-6}
10^2-10^3	1.9×10^{-4}	5.6×10^{-6}	1.4×10^{-7}

[a]Assumptions: 10−100 mg soil ingested per person per day for 300 days per year; 0.1−1 rotavirus and *Campylobacter*, and 0.01−0.1 *Cryptosporidium* oocyst, per 10^5 *E. coli*; no pathogen die-off; $N_{50} = 6.7 \pm 25\%$ and $\alpha = 0.253 \pm 25\%$ for rotavirus; $N_{50} = 896 \pm 25\%$ and $\alpha = 0.145 \pm 25\%$ for *Campylobacter*; and $r = 0.0042 \pm 25\%$ for *Cryptosporidium*.

[b]Soil quality (*E. coli* per 100 g) taken, as a worst-case scenario, as the wastewater quality (*E. coli* per 100 mL).

Source: WHO 2006b.

Annex A–Part 3
Quantitative Microbial Risk Analysis:
Developments since the 2006 WHO Guidelines

A3.1 Selection of a More Appropriate Value for the Maximum Tolerable Additional Burden of Disease

The first task in any health-risk assessment is to establish the maximum tolerable additional burden of disease – i.e., the maximum DALY loss per person per year (pppy). The 2006 WHO Guidelines use a value of 10^{-6} pppy for this (section A2.3), but is this the most appropriate value to use, especially in low-income countries?

The Guidelines [volume 2, section 4.5] state that:

> *Wastewater treatment may be considered to be of a low priority if the local incidence of diarrheal disease is high and other water-supply, sanitation and hygiene-promotion interventions are more cost-effective in controlling transmission. In such circumstances, it is recommended that, initially, a national standard is established for a locally appropriate level of tolerable additional burden of disease based on the local incidence of diarrheal disease – for example, $\leq 10^{-5}$ or $\leq 10^{-4}$ DALY [loss] per person per year* [emphasis added].

This position was re-affirmed by WHO (2007) for drinking water in *Levels of Protection*, which was originally published as part of its 'Rolling Revision' of the drinking-water quality guidelines, and by WHO (2010). *Levels of Protection*, which is now formally incorporated into the WHO Drinking-water Quality Guidelines (WHO 2008a), reads in full as follows:

> *Although these Guidelines employ a 10^{-6} DALY [loss] per person per year reference level of risk, it is important to recognize that this level of risk may not be achievable or realistic in some locations and circumstances. This is because the magnitude of microbial, chemical or radio-logical contamination sources, the levels of exposure to these sources from all exposure routes (water, food, air, direct personal contact, etc.) and **the overall disease burden from these sources may be such that setting a 10^{-6} DALY [loss] per person per year level of risk from waterborne exposure is neither realistically attainable nor consistent with the overall levels of risk from all sources of exposure**. That is, 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** [emphasis added].*

In accordance with the Stockholm Framework (Fewtrell and Bartram 2001), this should also be applied to wastewater use in agriculture. The 'harmony' of the Stockholm Framework is that the acceptable disease risk from all water-related exposures should be the same − i.e., the

tolerable additional burdens of disease from, for example, drinking fully-treated drinking water, from working in wastewater-irrigated fields, and from consuming wastewater-irrigated crops. Therefore, if a DALY loss of $\leq 10^{-x}$ pppy is acceptable for drinking fully-treated drinking water, then it is also acceptable for working in wastewater-irrigated fields and consuming wastewater-irrigated foods eaten uncooked.

This is really the key to the adoption of the 2006 Guidelines in developing countries since setting a tolerable maximum additional burden of disease of 10^{-4} DALY loss pppy, for example, means that the design disease risk and the design infection risk are both 2-log units higher, and the required pathogen reductions 2 log units lower, than for the 10^{-6} DALY loss pppy adopted as the 'default' value in the 2006 WHO Guidelines. However, it has to be decided whether a maximum tolerable additional DALY loss of 10^{-4} pppy acceptable or not.

Reasons in favor of a maximum tolerable additional DALY loss of 10^{-4} pppy

1. The reason why the 2006 Guidelines use this value of 10^{-6} DALY loss pppy is because, as noted in section A2.3, it is used in the third edition of the WHO *Drinking-water Quality Guidelines* (WHO, 2004, 2008a) since it corresponds very closely to the fatal waterborne 70-year lifetime cancer risk of 10^{-5} per person accepted by US EPA (Munro and Travis 1986). This 70-year risk of 10^{-5} per person is equivalent to an <u>annual</u> risk of 1.4×10^{-7} per person. Whether this is a reasonable level of acceptable risk can only be judged by knowing how many Americans die each year from cancer. Horner et al. (2009, Table 2.5) give the 2006 age•adjusted mortality rate from all causes of cancer for both sexes and all races as 181.07 per 100,000 population – i.e., an incidence of 1.8×10^{-3} pppy. Thus the fatal waterborne-cancer risk of 1.4×10^{-7} pppy is <u>four</u> orders of magnitude lower than the actual fatal all•cancer incidence of 1.8×10^{-3} pppy.

A DALY loss of 10^{-4} pppy would be equivalent to a fatal waterborne lifetime cancer risk of 10^{-3} per person and thus to an annual risk of $\sim 10^{-5}$ per person. This is two orders of magnitude lower than the actual fatal all•cancer incidence of $\sim 10^{-3}$ pppy – i.e., an additional DALY loss of 10^{-4} pppy would increase this by only $\sim 1\%$.

2. The current global incidence of diarrheal disease is extremely high: in order-of-magnitude terms it is 0.1−1 pppy (Table A3.1; see also Kosek et al. 2003). A tolerable diarrheal disease risk of 10^{-2} pppy, equivalent to a 10^{-4} DALY loss pppy, is 1−2 orders of magnitude lower than the current diarrheal-disease incidence. For an individual it is equivalent to an additional episode of diarrheal disease once every 100 years (essentially once per lifetime), which is hardly a matter of significant public health concern − see also Haas (1996, 8) who comments on US EPA's use of a waterborne-disease risk of 10^{-4} pppy as follows:

> *It is becoming apparent that some key factors used for computing the 1:10,000 level of acceptable risk may not be correct. ... the total burden of waterborne illness associated with current water treatment practice in the Ubnited States may be as high as several million cases per year. This would translate to an annual illness rate of perhaps 1:100, suggesting that the current benchmark* [of 1:10,000] *may be far too stringent.*

Table A3.1. Diarrheal disease (DD) incidence per person per year in 2000

World region	DD incidence in all ages	DD incidence in 0−4 year olds	DD incidence in 5−80+ year olds
Industrialized countries[a]	0.2	0.2–1.7	0.1–0.2
Developing countries	0.8–1.3	2.4–5.2	0.4–0.6
Global average	0.7	3.7	0.4

[a] In some industrialized countries diarrheal disease incidence is much higher − for example, 0.92 pppy for 'infectious gastroenteritis' in Australians of all ages (Hall et al., 2005) and 0.79 pppy for 'acute gastroenteritis' in Americans of all ages (Mead et al., 1999) − i.e., in the developing-country range shown in the table.

Source: Mathers et al. 2002.

3. In low- and middle-income countries diarrheal diseases caused a total DALY loss of 59 million in 2001 (Lopez et al. 2006, Ezzati et al. 2006). Thus in 2001, for the then total developing-country population of 5,615 millions (UNFPA 2002), the DALY loss due to diarrheal diseases was:

$$\frac{59\,\text{million DALYs lost per year}}{5,615\,\text{million people}} = \sim 0.0105 \text{ pppy}$$

An additional DALY loss of 10^{-4} pppy would increase this to 0.0106 pppy – i.e., an increase of just under 1%. Such an increase is not epidemiologically significant (and, in any case, would be extremely difficult to detect).

Thus it seems perfectly reasonable to accept a maximum additional DALY loss of 10^{-4} pppy for wastewater use in agriculture. The required pathogen reductions would then be:
- To protect fieldworkers' health (restricted irrigation, labor-intensive agriculture): 2 log units, and
- To protect crop consumers' health (unrestricted irrigation): 4−5 log units.

The implications of these required pathogen reductions are:
- Wastewater treatment processes to achieve a pathogen reduction of only 2 log units are very simple and very low-cost, and
- Reliable post-treatment health-protection control measures (Table A2.2) can easily achieve the remaining 2−3-log unit reduction required for unrestricted irrigation − for

example, pathogen die-off (which can be expected to achieve a reduction of at least 2 log units in warm climates) and produce washing in clean water (1 log unit).

A3.2 Improved Method for Estimating Annual Infection Risks

Since the publication of the Guidelines, an improved method of estimating annual infection risks from QMRA-MC simulations has been developed by Karavarsamis and Hamilton (2010), as follows:

1. Using the appropriate dose-response equation (section A2.1) in an appropriate QMRA-Monte Carlo computer program, an estimate of median annual infection risk is determined by a Monte Carlo simulation in which the number of iterations is set equal to the number of days of exposure per year.

2. This is repeated 9,999 times, so that there are 10,000 estimates of median annual infection risk.

3. The median and 95-percentile values of these 10,000 estimates are then calculated in order to provide a much more robust estimate of median and 95-percentile annual infection risks.

Thus the program determines 10,000 estimates of median annual risk based on what happens in any one <u>year</u> of exposure (i.e., *n* exposures to a pathogen dose *d*), rather than (as in the procedure used in the Guidelines) a much less robust estimate of median annual risk determined from 10,000 estimates of annual risk based on what happens on any one <u>day</u> of exposure. This approach results in similar values for estimates of median annual risks, but much lower estimates for 95-percentile annual risks, and there is less 'spread' of results (Table A3.2).

Table A3.2. Unrestricted irrigation: comparison of the Karavarsamis-Hamilton method and the method used in the WHO Guidelines for estimating annual rotavirus infection risks from the consumption of wastewater-irrigated lettuce by 10,000 Monte Carlo simulations [a]

Wastewater quality (*E. coli* numbers per 100 mL)		Rotavirus infection risk pppy estimated by the method of Karavarsamis and Hamilton (2010)	Rotavirus infection risk pppy estimated by the method used in the 2006 WHO Guidelines
10^3-10^4	Median risk:	0.36	0.30
	95%-ile risk:	0.39	0.71
	Minimum:[b]	0.30	1.1×10^{-2}
	Maximum:[b]	0.44	0.97
100−1000	Median risk:	4.5×10^{-2}	3.5×10^{-2}
	95%-ile risk:	4.9×10^{-2}	0.11
	Minimum:	3.5×10^{-2}	1.0×10^{-3}
	Maximum:	5.5×10^{-2}	0.27
10−100	Median risk:	4.6×10^{-3}	3.5×10^{-3}
	95%-ile risk:	5.0×10^{-3}	1.2×10^{-2}
	Minimum:	3.5×10^{-3}	9.5×10^{-5}
	Maximum:	5.7×10^{-3}	3.0×10^{-2}

[a]Assumptions: 100 g lettuce eaten per person per 2 days; 10−15 mL wastewater remaining on 100 g lettuce after irrigation; 0.1−1 rotavirus per 10^5 *E. coli*; no pathogen die-off; $N_{50} = 6.7 \pm 25\%$ and $\alpha = 0.253 \pm 25\%$.

[b]The lowest and highest values of the 10,000 risk simulations.

Table A3.3. Restricted irrigation: labor-intensive agriculture with exposure for 300 days per year − median infection risks from involuntary ingestion of wastewater-contaminated soil estimated by 10,000 Karavarsamis-Hamilton Monte Carlo simulations[a]

Soil quality (*E. coli* per 100 g)[b]	Infection risk per person per year		
	Rotavirus	*Campylobacter*	*Cryptosporidium*
10^7−10^8	1	0.70	2.1×10^{-2}
10^6−10^7	0.96	0.12	2.1×10^{-3}
10^5−10^6	0.28	1.3×10^{-2}	2.1×10^{-4}
10^4−10^5	3.3×10^{-2}	1.3×10^{-3}	2.1×10^{-5}
10^3−10^4	3.3×10^{-3}	1.3×10^{-4}	2.1×10^{-6}

[a]Assumptions: 10−100 mg soil ingested per person per day for 300 days per year; 0.1−1 rotavirus and *Campylobacter*, and 0.01−0.1 *Cryptosporidium* oocyst, per 10^5 *E. coli*; N_{50} = 6.7 ± 25% and α = 0.253 ± 25% for rotavirus; N_{50} = 896 ± 25% and α = 0.145 ± 25% for *Campylobacter*; r = 0.0042 ± 25% for *Cryptosporidium*. No pathogen die-off.

[b]Soil quality taken, as a worst-case scenario, as the wastewater quality.

Table A3.4. Unrestricted irrigation: median infection risks from the consumption of wastewater-irrigated lettuce estimated by 10,000 Karavarsamis-Hamilton Monte Carlo simulations[a]

Wastewater quality (*E. coli* per 100 mL)	Infection risk per person per year		
	Rotavirus	*Campylobacter*	*Cryptosporidium*
$10^7 - 10^8$	1	1	0.94
...			
$10^3 - 10^4$	0.37	1.7×10^{-2}	2.9×10^{-4}
$100 - 1000$	4.4×10^{-2}	1.7×10^{-3}	2.9×10^{-5}
$10 - 100$	4.6×10^{-3}	1.8×10^{-4}	2.9×10^{-6}
$1 - 10$	4.6×10^{-4}	1.7×10^{-5}	2.9×10^{-7}

[a]Assumptions: 100 g lettuce eaten per person per 2 days; 10−15 mL wastewater remaining on 100 g lettuce after irrigation; 0.1−1 rotavirus and *Campylobacter*, and 0.01−0.1 *Cryptosporidium* oocyst, per 10^5 *E. coli*; no pathogen die-off; $N_{50} = 6.7 \pm 25\%$ and $\alpha = 0.253 \pm 25\%$ for rotavirus; $N_{50} = 896 \pm 25\%$ and $\alpha = 0.145 \pm 25\%$ for *Campylobacter*; $r = 0.0042 \pm 25\%$ for *Cryptosporidium*.

Tables A3.3 and A3.4 detail the QMRA-MC estimates of infection risk for restricted and unrestricted irrigation, respectively, in the same way as determined in the 2006 WHO Guidelines (Tables A2.3 and A2.5), but with two important differences: (a) use of the Karavarsamis-Harrison method, and (b) without any pathogen die-off. The implications are:

- *Restricted irrigation:* if a maximum tolerable DALY loss of 10^{-4} pppy is adopted for fieldworkers (rather than the 10^{-6} DALY loss pppy used in the Guidelines), the tolerable rotavirus infection risk is $\sim 10^{-1}$ and a pathogen reduction of 2 log units is required, which has to be achieved by wastewater treatment.

- *Unrestricted irrigation:* if a maximum tolerable DALY loss of 10^{-4} pppy is also adopted for consumers, and thus a tolerable rotavirus infection risk of $\sim 10^{-1}$, then a pathogen reduction of 4 log units is required (from $10^7 - 10^8$ per 100 ml to $10^3 - 10^4$ per 100 ml), which could be achieved by wastewater treatment (2 log units, as for restricted irrigation) and, for example, die-off (2 log units) (*cf.* Table A2.2).

A3.3. Unrestricted Irrigation: Norovirus Infection Risks

Recently it has become possible to use QMRA-Monte Carlo techniques to estimate norovirus infection risks (Mara and Sleigh 2010a,c). The dose-response data needed to do this were only published in 2008 (Teunis et al. 2008), so it was not possible for norovirus to have been considered in the 2006 WHO Guidelines.

Norovirus (NV, formerly called Norwalk or Norwalk-like virus) is the major viral pathogen causing diarrheal disease in adults − in contrast rotavirus mainly affects children under 5, and commonly under 2, years of age, although NV does cause diarrhea in children (Patel et al. 2008). It is therefore a better reference viral pathogen than rotavirus for wastewater-use studies as young children are less exposed than adults, either as field-workers (although they may play in wastewater-irrigated fields while their mothers work in them) or as consumers (children under 2, especially, eat little wastewater-irrigated foods). That norovirus is a more suitable reference viral pathogen than rotavirus is illustrated by the fact that in the USA during 1998−2007 there were 1,773 confirmed foodborne norovirus outbreaks, but only 4 confirmed foodborne rotavirus outbreaks (CDC 2009).

Using a DALY loss per case of 9×10^{-4} per case of NV disease (Kemmeren *et al*. 2006) and an NV disease/infection ratio of 0.8 (Moe 2009), the tolerable NV disease and infection risks corresponding to a tolerable DALY loss of 10^{-4} pppy are:

$$\text{Tolerable NV disease risk} = \frac{\text{Tolerable DALY loss pppy}}{\text{DALY loss per case of NV disease}} = \frac{10^{-4}}{9 \times 10^{-4}} = 0.11 \text{ pppy}$$

$$\text{Tolerable NV infection risk} = \frac{\text{Tolerable NV disease risk pppy}}{\text{NV disease/infection ratio}} = \frac{0.11}{0.8} = 0.14 \text{ pppy}$$

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. The resulting estimates of median risk obtained, using the same assumptions as for the index pathogens in Table A3.4, are given in Table A3.5, which shows that a reduction of 4 log units results in an NV infection risk of 0.25 pppy, which is only marginally higher than the tolerable NV infection risk of 0.14 pppy determined above. Table A3.5 also includes, for comparison, rotavirus infection risks – these are broadly similar to the norovirus infection risks.

Lettuce consumption in urban Ghana: norovirus infection risks

Exposure to wastewater pathogens present in wastewater-irrigated foods varies with 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 ~10−12 g of lettuce in ready-to-eat street-vended food on each of four days per week.[37] NV infection risks for a DALY loss of 10^{-4} pppy and for this Ghanaian consumption of lettuce were determined by 10,000 Karavarsamis-Hamilton Monte Carlo simulations for various wastewater qualities (Table A3.6). A 3-log unit NV reduction achieves an NV infection risk of 0.3 pppy, which is a little higher than the tolerable NV infection risk of 0.13 pppy. However, a 4-log unit reduction is feasible as it could be simply achieved by treatment (1 log unit) and produce disinfection (3 log unit) (*cf.* Table A2.2).

Parameters used in QMRA risk simulations

Table A3.7 lists the parameters used in QMRA risk simulations and gives recommendations for the ranges to be used for each.

---

[37] This refers to a specific situation in one developing country and may or may not be representative of what happens elsewhere; however, it is much less than the 100 g of lettuce consumed on alternate days used by Shuval et al. (1997) to reflect the situation in Israel (this value of 100 g per 2 days was also used in the 2006 WHO Guidelines).

Table A3.5. Unrestricted irrigation: median norovirus and rotavirus infection risks per person per year from the consumption of 100 g of wastewater-irrigated lettuce every two days estimated by 10,000 Karavarsamis-Hamilton Monte Carlo simulations[a]

Wastewater quality (*E. coli* per 100 mL)	Median norovirus infection risk pppy	Median rotavirus infection risk pppy
$10^7–10^8$	1	1
…		
$10^3–10^4$	0.25	0.36
100−1000	2.9×10^{-2}	4.5×10^{-2}
10−100	2.9×10^{-3}	4.6×10^{-3}

[a]Assumptions: 10−15 mL wastewater remaining on 100 g lettuce after irrigation; 0.1−1 norovirus per 10^5 *E. coli*; no pathogen die-off; $N_{50} = 6.7 \pm 25\%$ and $\alpha = 0.253 \pm 25\%$ for rotavirus; and dose-response data (Teunis et al. 2008) $\pm 25\%$ for norovirus.
Source: Mara and Sleigh 2010a.

Table A3.6. Unrestricted irrigation: median norovirus infection risks per person per year from the consumption of 10−12 g of wastewater-irrigated lettuce on four occasions per week estimated by 10,000 Karavarsamis-Hamilton Monte Carlo simulations[a]

Wastewater quality (*E. coli* per 100 mL)	Median norovirus infection risk pppy
$10^7–10^8$	1
…	
$10^4–10^5$	0.31
$10^3–10^4$	3.6×10^{-2}

[a]Assumptions: 10−15 mL wastewater remaining on 100 g lettuce after irrigation; 0.1−1 norovirus per 10^5 *E. coli*; no pathogen die-off; dose-response data (Teunis et al. 2008) $\pm 25\%$.
Source: Mara and Sleigh 2010a.

Table A3.7. Parameters used in QMRA-MC risk simulations

Parameter	Units	Notes
E. coli (or fecal coliform) count in wastewater (unrestricted irrigation) or in soil (restricted irrigation)	per 100 mL or per 100 g	Run the QMRA-MC program for single-log ranges (10^x–10^{x+1})—e.g., for unrestricted irrigation, from 10^7–10^8 (raw wastewater) to 1–10 ("Californian" treated wastewater)
Number of noroviruses per 10^5 *E. coli*	n.a.	Typical range: 0.1–1.
Wastewater remaining on 100 g crop after irrigation (unrestricted irrigation)	mL	Value depends on crop – e.g., 10–15 mL for lettuce, 1–5 mL for onions[a]
Quantity of crop consumed per person (unrestricted irrigation)	g per exposure event	Use local values[b]
Quantity of soil ingested per person (restricted irrigation)	mg per day	Use, for example, 10–100 mg per day for labor-intensive agriculture, and 1–10 mg per day for highly mechanized agriculture
Exposure (every *n* days for unrestricted irrigation, or *n* days per year for restricted irrigation)	n.a.	For unrestricted irrigation this could be a fixed value—e.g., 2 days, as used by Shuval et al. (1997), or a range—e.g., 3–5 days for someone who eats a sandwich on 3–5 days per week.
Pathogen reduction factor [die-off]	log units	Set range to 0–0 for die-off to be excluded from QMRA-MC risk simulations.
Disease/infection ratio	n.a	Set range to 1–1 for infection risk to equal disease risk (i.e., to determine norovirus infection risks).
Variation of dose-response data from value in dataset	±%	Commonly ±25%. This allows for some uncertainty in the dose-response dataset.
Number of simulations in each QMRA-MC run	n.a.	Usually 10,000 or 1000.

[a]Shuval et al. 1997 found mean values of 10.8 mL per 100 g lettuce and 0.4 mL per 100 g cucumbers. Hamilton 2005 (quoted in EPHC/NRMMC/AHMC 2006) reported mean values of 3.3–8.9 mL per 100 g cabbages (three types) and 1.9 mL per 100 g broccoli.
[b]Shuval et al. 1997 used a fixed value of 100 g in Israel; Seidu et al. 2008 used a range of 10–12 g in Ghana.

A3.4. Unrestricted Irrigation: *Ascaris* Infection Risks

Ascaris lumbricoides parasitizes ~1.2 billion people in developing countries (de Silva et al. 2003). Female *Ascaris* worms produce ~200,000 eggs per day and, although most *Ascaris* eggs only survive for a few weeks on crop surfaces, some remain viable for several months and so represent a risk to health when they are ingested with wastewater-irrigated foods eaten uncooked (Strauss 1985). It would be very useful to be able to quantify *Ascaris* risks as its properties (pathogenicity, development and survival in the environment) are very different from those of viral and bacterial pathogens, despite it having an overall feco-oral environmental transmission route.

Ascaris dose-response data were published only in 2009 (Navarro et al. 2009), so it was not possible to have used *Ascaris* as a reference helminthic pathogen in the 2006 WHO Guidelines. Even though the 2006 WHO guideline value for helminth eggs of ≤1 egg per liter of treated wastewater is based on epidemiological data, it is nevertheless very useful to be able to determine required log unit reductions of *Ascaris* (which is generally the commonest helminth and the eggs of which are able to survive for very long periods of time in the environment) by QMRA and thus to split these between wastewater treatment and post-treatment health-protection control measures (Table A2.2), as is done for viral, bacterial and protozoan pathogens, since this allows a lower level of wastewater treatment.

For a tolerable DALY loss of 10^{-4} pppy, a DALY loss per case of ascariasis of 8.25×10^{-3} (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:

$$\frac{\text{Tolerable DALY loss pppy}}{\text{DALY loss per case of ascariasis}} = \frac{10^{-4}}{8.25 \times 10^{-3}} = 1.2 \times 10^{-2} \text{ pppy}$$

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-Hamilton method and using the values of N_{50} and α determined by Navarro et al. (2009) for the beta-Poisson equation (these authors found that the beta-Poisson equation was better for *Ascaris* than the exponential equation). The resulting estimates of median *Ascaris* infection risk are given in Table A3.8, which shows that 1 egg per litre results in an *Ascaris* infection risk of ~6×10^{-3} pppy, which is just below the tolerable *Ascaris* infection risk of ~10^{-2} pppy determined above. Thus, in ascariasis-hyperendemic areas (~1000 eggs per litre of raw wastewater) a 3-log unit reduction of *Ascaris* eggs is required. This could be achieved, for example, by a 1-log unit reduction by treatment and a 2-log unit reduction by produce peeling (*cf*. Table A2.2).

Table A3.8. Unrestricted irrigation: median *Ascaris* infection risks for children under 15 from the consumption of raw wastewater-irrigated carrots estimated by 10,000 Karavarsamis−Hamilton Monte Carlo simulations[a]

Number of *Ascaris* eggs per litre of wastewater	Median *Ascaris* infection risk pppy	Notes
100–1000	0.86	Raw wastewaters in hyperendemic areas.
10–100	0.24	Raw wastewaters in endemic areas.
1–10	2.9×10^{-2}	Treated wastewaters.
1	5.5×10^{-3}	Wastewater quality required to comply with the 1989 and 2006 WHO Guidelines.
0.1–1	3.0×10^{-3}	Highly treated wastewaters.
0.1	5.5×10^{-4}	Wastewater quality recommended by Blumenthal *et al*. (2000) to protect children under 15.
0.01–0.1	3.0×10^{-4}	Treated wastewaters in non-endemic areas.

[a]Assumptions: 30–50 g raw carrots consumed per child per week; 3–5 mL wastewater remaining on 100 g carrots after irrigation; $N_{50} = 859 \pm 25\%$ and $\alpha = 0.104 \pm 25\%$; no *Ascaris* die-off .

Source: Mara and Sleigh 2010b.

A3.5. Restricted Irrigation: Norovirus Infection Risks

In the 2006 Guidelines health risks for restricted irrigation were estimated by assuming the fieldworkers involuntarily ingested wastewater-contaminated soil. An ingestion of 10−100 mg of soil per day for 300 days per year was used to represent labor-intensive agriculture in developing countries. With hygiene education it is not infeasible to postulate that the quantity of soil ingested can be substantially reduced, perhaps to 1−10 mg per day. Table A3.9, which gives norovirus infection risks for this quantity of soil ingestion for 300 days per year, shows that a norovirus reduction of 1 log unit results in a norovirus infection risk of 0·32 pppy (i.e., one episode of norovirus diarrhoea every three years), which is higher than the tolerable norovirus infection risk of 0·13 pppy determined in section A3.3 (one episode of norovirus diarrhoea every seven years), but acceptable if combined with sustained hygiene education and the provision of (a) hand-washing facilities on or adjacent to the site being irrigated, and (b) oral rehydration salts/solutions whenever required.

Table A3.9. Restricted irrigation: median norovirus infection risks from the ingestion of 1−10 mg of wastewater-saturated soil per day for 300 days per year estimated by 10,000 Karavarsamis-Hamilton Monte Carlo simulations [a]

Noroviruses per 100 g soil	Median norovirus infection risk pppy
100−1000	0.98
10−100	0·32
1−10	$3\cdot7 \times 10^{-2}$

[a]Assumptions: soil quality per 100 g taken, as a worst-case scenario, as the wastewater quality per 100 mL; 10^5 noroviruses per 10^5 *E. coli*; no pathogen die-off; dose-response data (Teunis et al. 2008) ± 25%.

Source: Mara and Sleigh 2010c.

A3.6 Restricted Irrigation: *Ascaris* Infection Risks

Table A3.10 gives the equivalent calculations for *Ascaris* as given in Table A3.9 for norovirus. It shows that, in areas where ascariasis is hyperendemic, a 1-log unit reduction results in an ascariasis risk of $1\cdot5 \times 10^{-2}$ pppy, which is close to the tolerable ascariasis risk of $1\cdot2 \times 10^{-2}$ pppy determined in section A3.4.

Table A3.10. Restricted irrigation: median *Ascaris* infection risks from the ingestion of 1−10 mg of wastewater-saturated soil per day for 300 days per year estimated by 10,000 Karavarsamis-Hamilton Monte Carlo simulations [a]

Ascaris eggs per kg soil	Median *Ascaris* infection risk pppy
100−1000	0·14
10−100	1.5×10^{-2}
1−10	$1\cdot5 \times 10^{-3}$

[a]Assumptions: soil quality (eggs per kg) taken, as a worst-case scenario, as the wastewater quality (eggs per liter); $N_{50} = 859 \pm 25\%$ and $\alpha = 0\cdot104 \pm 25\%$; no pathogen die-off.

Source: Mara and Sleigh 2009c.

Availability of QMRA-MC computer programs

All the QMRA-Monte Carlo computer programs referred to in this Annex are freely available, together with User Guides, at:

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* (or fecal coliform) 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 would determine pathogen risks directly (as done, in Table A3.9 for norovirus), rather than as a range of *E. coli* numbers per 100 mL.

A "QMRA Beginner's Guide" is available at:

http://www.personal.leeds.ac.uk/~cen6ddm/QMRAbeginners.html

Annex A–Part 4

Verification Monitoring of Pathogen Reductions Achieved by Wastewater Treatment and Health-protection Control Measures

A4.1 Wastewater Treatment

It is not generally possible in developing countries, nor in most non-research situations in industrialized countries, to undertake routine monitoring of viral, bacterial and protozoan pathogens in treatment-plant effluents. Recourse has to be made to the use of indicator bacteria, typically fecal coliforms (section A4.2).

Helminth eggs can be counted in raw and treated wastewaters, although the enumeration technique is not straightforward. If waste stabilization ponds are used for wastewater treatment, then the mean hydraulic retention time in the ponds can be used to calculate egg removal efficiencies (section A4.3).

A4.1.1 Indicator bacteria

Fecal coliforms are the most commonly used indicator bacteria, and they are very easy to count using a very simple 5-tube most-probable-number (MPN) technique – full details of how to conduct this test are given in Ayres and Mara (1996).

If the QMRA-MC risk simulations show that wastewater treatment should achieve a 2-log unit pathogen reduction (see section A3.2), then the fecal coliform count has to be reduced from 10^7–10^8 per 100 mL to 10^5–10^6 per 100 mL, although a prudent designer might design the treatment plant to achieve a 3-log unit reduction of fecal coliforms (i.e., from 10^7–10^8 per 100 mL to 10^4–10^5 per 100 mL), given the uncertainty of the relationship between pathogen and fecal coliform numbers and how this might change through the treatment plant. Regular (perhaps weekly or fortnightly, but certainly monthly) monitoring would be required to show whether or not the effluent from the treatment plant contained $\leq 10^5$ (or $\leq 10^4$) fecal coliforms per 100 mL.

A4.1.2 Helminth egg removal in waste stabilization ponds

Waste stabilization ponds (WSP) (Mara 2004, Shilton 2006) are currently the only wastewater treatment process that can be reliably designed for helminth egg removal (Ayres et al. 1992). The design equation is:

$$R = 100[1 - 0.41\exp(-0.49\theta + 0.0085\theta^2)] \tag{A4.1}$$

where R is the percentage egg removal in a WSP which has a mean hydraulic retention time of θ days. Thus, if the number of eggs in the raw wastewater (E_i eggs per liter) and θ are known, the number of eggs in the pond effluent (E_e eggs per liter) can be determined.

For a series of WSP comprising an anaerobic pond, a facultative pond and n equally-sized maturation ponds E_e is given by:

$$E_e = E_i(1 - r_a)(1 - r_f)(1 - r_m)^n \qquad\qquad (A4.2)$$

where $r = R/100$ and the subscripts a, f and m refer to the anaerobic, facultative and maturation ponds, respectively – see 'Worked Example A' below.

Equation A4.2 can be rearranged as follows:

$$E_i = \frac{E_e}{(1 - r_a)(1 - r_f)(1 - r_m)^n} \qquad\qquad (A4.3)$$

Equation A4.3 is useful because it permits the WSP operator to determine the maximum number of eggs in the raw wastewater that the ponds can remove down to any required level of E_e eggs per liter − see 'Worked Example B' below.

Worked Example A: Known number of eggs per liter of raw wastewater

Suppose (a) the raw wastewater to be used for unrestricted irrigation contains 1000 eggs per liter; (b) QMRA-MC risk simulations have shown that a total *Ascaris* egg reduction of 3 log units is required for unrestricted irrigation (*cf.* Table A3.8); and (c) it has been decided that this is to be achieved by (i) wastewater treatment in a WSP system comprising a 1-day anaerobic pond and a 5-day facultative pond, and (ii) produce washing in clean water, which achieves a 1-log unit *Ascaris* reduction (*cf.* Table A2.2). Thus wastewater treatment in the WSP has to achieve a 2-log unit *Ascaris* reduction and therefore the effluent from the facultative pond has to contain ≤10 eggs per liter. From equation A4.1 r_a $(=R_a/100) = 0.75$ and $r_f = 0.96$ [$r_m = 0$ as there are no maturation ponds], and therefore from equation A4.2:

$$E_e = E_i(1 - r_a)(1 - r_f) = 1000(1 - 0.75)(1 - 0.96) = 10 - satisfactory.$$

Verification monitoring can thus simply be based on measurements of helminth eggs in the raw wastewater (see Ayres and Mara 1996) and the values of the WSP retention times in the irrigation season (these are given by V/Q, where V is the pond volume in m³ and Q is the wastewater flow in m³/day). This is very advantageous as counting the number of eggs per liter of raw wastewater is much easier than counting egg numbers of ~10 per liter and below.

Worked Example B: Unknown number of eggs per liter of raw wastewater

Suppose that the exact number (or range of numbers) of eggs per liter of the raw wastewater is unknown, but that it is known that local raw wastewaters 'never' contain more than around 200−500 eggs per liter. Then, from equation A4.3, for the 1-day anaerobic pond and the 5-day facultative pond to produce an effluent with ≤10 eggs per liter:

$$E_i = \frac{E_e}{(1 - r_a)(1 - r_f)} = \frac{10}{(1 - 0.75)(1 - 0.96)} = 1000 \text{ eggs per liter}$$

Thus there would be little need for verification monitoring – perhaps only once or twice during the irrigation season – as the number of eggs in the raw wastewater that the WSP system can cope with (i.e., without exceeding the required effluent quality) is much higher than the numbers of eggs commonly encountered in the raw wastewater.

A4.2 Health-protection control measures

To assess the efficacy of the health-protection control meaures listed in Table A2.2 a Hazard Analysis Critical Control Point (HACCP) system should be established. HACCP systems consist of the following seven principles (FAO, 2003):

1. Conduct a hazard analysis.
2. Determine the Critical Control Points (CCPs).
3. Establish critical limit(s).
4. Establish a system to monitor control of the CCP.
5. Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.
6. Establish procedures for verification to confirm that the HACCP system is working effectively.
7. Establish documentation concerning all procedures and records appropriate to these principles and their application.

Principles 1−4 for unrestricted irrigation:

Principle 1: HACCP is applied to both the wastewater treatment plant and each of the post-treatment health-protection control measures in operation since if one of them performs sub-optimally or fails completely, it becomes a health hazard.

Principle 2: The number of CCPs corresponds to the wastewater treatment plant effluent and each post-treatment health-protection control measures in operation.

Principle 3: The critical limit of each CCP is the number of *E. coli* (or fecal coliforms) which shows unequivocally that the design removal for the CCP is being achieved. In the case of pathogen die-off (x log units per day) it is not possible to determine the time interval between the last irrigation and consumption (as the food may be kept refrigerated at home for a few days before it is consumed), but it is possible to monitor the time interval between the last irrigation and the appearance of the food in local shops and supermarkets.[38]

Principle 4: The system to monitor control of the CCP comprises sampling and microbiological analysis at an established frequency (e.g., weekly or fortnightly, certainly not less than monthly).

Since HACCP for unrestricted irrigation is solely concerned with the safety of wastewater-irrigated foods, principally those eaten uncooked, it is worth noting that ready-to-eat foods (such as prepared sandwiches and salads on sale in local shops and supermarkets) are deemed to be of 'acceptable' quality in the United Kingdom if they contain $<10^4$ *E. coli* per 100 g (Gilbert et al. 2000).[39] Thus if lettuce, which is a common constituent of many ready-to-eat

---

[38] This is routinely done, for example, in Australia (EPHC/NRMMC/AHMC 2006).

[39] This guideline value is used in many other countries, including Australia, Canada and New Zealand (Institute of Medicine 2003).

foods, can contain up to 10^4 *E. coli* per 100 g, then it is clearly not necessary to irrigate it with a treated wastewater containing <10^4 *E. coli* per 100 mL, especially as post-treatment die-off always occurs. This illustrates the inherent problem with the 1989 WHO Guidelines which require ≤1000 fecal coliforms per 100 mL for unrestricted irrigation, and the inherent advantage of the Australian National and the 2006 WHO Guidelines which permit the inclusion of pathogen reductions that occur post-treatment.