Health risks in wastewater irrigation: Comparing estimates from quantitative microbial risk analyses and epidemiological studies

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ABSTRACT

The combination of standard quantitative microbial risk analysis (QMRA) techniques and 10,000-trial Monte Carlo risk simulations was used to estimate the human health risks associated with the use of wastewater for unrestricted and restricted crop irrigation. A risk of rotavirus infection of 10^{-2} per person per year (pppy) was used as the reference level of acceptable risk. Using the model scenario of involuntary soil ingestion for restricted irrigation, the risk of rotavirus infection is $\sim 10^{-2}$ pppy when the wastewater contains $\leq 10^6$ *Escherichia coli* per 100 ml and when local agricultural practices are highly mechanised. For labour-intensive agriculture the risk of rotavirus infection is $\sim 10^{-2}$ pppy when the wastewater contains $\leq 10^5$ *E. coli* per 100 ml; however,, the wastewater quality should be $\leq 10^4$ *E. coli* per 100 ml when children under 15 are exposed. With the model scenario of lettuce consumption for unrestricted irrigation, the use of wastewaters containing $\leq 10^4$ *E. coli* per 100 ml results in a rotavirus infection risk of $\sim 10^{-2}$ pppy; however, again based on epidemiological evidence from Mexico, the current WHO guideline level of $\leq 1,000$ *E. coli* per 100 ml should be retained for root crops eaten raw.

Key words | epidemiology, health, irrigation, risk analysis, rotavirus, wastewater

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INTRODUCTION

In 1989 the World Health Organization published guidelines for the microbiological quality of treated wastewaters used in agriculture and aquaculture (WHO 1989). The guidelines for agricultural use were: (a) for restricted irrigation (i.e. the irrigation of all crops except salad crops and vegetables that may be eaten uncooked), ≤ 1 human intestinal nematode egg l^{-1} (the nematodes are the human roundworm, *Ascaris lumbricoides*; the human whipworm, *Trichuris trichiura*; and the human hookworms, *Ancylostoma duodenale* and *Necator americanus*); and (b) for unrestricted irrigation (i.e. including the irrigation of salad crops and vegetables eaten uncooked), the same nematode egg guideline and $\leq 1,000$ faecal coliforms (FC) per 100 ml.

These guidelines, particularly the FC guideline, caused considerable controversy (for example, Shelef 1991), especially when compared with the standard of ≤ 2.2 total coliforms per 100 ml required by the State of California (1978) and with the later recommendation of the US Environmental Protection Agency and the US Agency for International Development for 'undetectable' (i.e. zero) FC per 100 ml (USEPA & USAID 1992, 2004). The FC guideline value of $\leq 1,000$ per 100 ml, originally introduced in the Engelberg Report (IRCWD 1985), had, at the time of its introduction, no rigorous epidemiological basis. However, it was known that (a) irrigation of salad crops with untreated wastewater caused excess disease in those who consumed

them (Shuval *et al.* 1986); (b) *Salmonella* occurrence was almost 100% in irrigation waters containing > 1,000 FC per ml (Geldreich & Bordner 1971); and (c) USEPA (1973) had recommended an FC standard of ≤ 1,000 per 100 ml of river waters used for unrestricted irrigation. Furthermore there had been no report of an outbreak of disease resulting from the use of treated wastewater for unrestricted irrigation. The WHO/Engelberg guidelines were defended later, but only in general terms (Shuval 1988; Mara 1995).

Since the publication of the WHO guidelines in 1989, the health risks of wastewater use in agriculture have been investigated in greater detail in two separate areas of research: quantitative microbial risk analysis (QMRA) applied to unrestricted irrigation (Shuval *et al.* 1997; Tanaka *et al.* 1998) and epidemiology (Blumenthal & Peasey 2003). In this paper we present the results of our QMRA applied to both restricted and unrestricted irrigation with wastewaters of various qualities (expressed in terms of numbers of *E. coli* per 100 ml; we use *E. coli* rather than FC as it is exclusively faecal in origin, although we use FC when referring to data based on this parameter). We then compare these results with those from recent epidemiological studies, and we also consider the implications of our results for the new WHO guidelines.

QUANTITATIVE MICROBIAL RISK ANALYSIS

Risk of infection or risk of disease?

Epidemiological studies can determine either the excess prevalence of infection (as measured by the proportion of infected or seropositive individuals in an exposed group compared with that of those in a control group), or the excess prevalence or incidence of disease (occurring during a specified time period) in an exposed group compared with a control group. In the present context individuals eating wastewater-irrigated salad crops, or working (or playing) in wastewater-irrigated fields, represent the exposed group, and those not eating such crops, or not working (or playing) in wastewater-irrigated fields, the control group. QMRA estimates the risk of infection in an exposed group, and this can be extended to estimate the risk of disease in that group by knowing (or making an assumption about) the likely proportion of infected individuals who develop the disease.

We used the combination of standard QMRA techniques (Haas *et al.* 1999) and 10,000-trial Monte Carlo simulations (Sleigh & Mara 2003) to estimate risks of infection from model pathogen-ingestion scenarios for both restricted and unrestricted irrigation. We then compared excess incidences of diarrhoeal disease determined from epidemiological wastewater-use field studies with the QMRA/Monte Carlo-simulated risks based on parameter values closer to those likely to occur in the field situations where the epidemiological studies were done.

Dose-response models

The dose-response models used were the β-Poisson model for rotavirus and *Campylobacter* infections and the exponential model for *Cryptosporidium* infection (Haas *et al.*, 1999). The equations are:

(a) β-Poisson dose-response model

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

(b) Exponential dose-response model

$$P_I(d) = 1 - \exp(-rd) \tag{2}$$

(c) Annual risk of infection

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

where $P_I(d)$ is the risk of infection in an individual exposed to (here, following ingestion of) a single pathogen dose d; $P_{I(A)}(d)$ is the annual risk of infection in an individual from n exposures per year to the single pathogen dose d; ID_{50} is the median infective dose; and α and r are pathogen 'infectivity constants'. For rotavirus $ID_{50} = 6.17$ and $\alpha = 0.253$; for *Campylobacter* $ID_{50} = 896$ and $\alpha = 0.145$; and for *Cryptosporidium* $r = 0.0042$ (Haas *et al.* 1999). $P_{I(A)}(d)$ can also be interpreted as the risk over a shorter (or longer) period: for example, an m-month risk with n now equal to the number of exposures during m months.

The value of $P_{I(A)}(d)$ is in the range 0–1. If $P_{I(A)}(d) = 1$, infection is certain. However, QMRA cannot determine whether an individual becomes infected more than once per year. Such information can only be found by epidemiological studies.

Exposure scenarios

Restricted irrigation

The model scenario we developed for restricted irrigation is the involuntary ingestion of soil particles by those working, or by young children playing, in wastewater-irrigated fields. This is a likely scenario as wastewater-saturated soil would contaminate the workers' or children's 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). We chose to investigate two 'sub-scenarios': (a) highly mechanized agriculture and (b) labour-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. Different soil ingestion ranges and numbers of exposure days per year were used for these two sub-scenarios.

We estimated median risks per person per year for rotavirus, *Campylobacter* and *Cryptosporidium* infections resulting from the ingestion of 1–10 mg of wastewater-contaminated soil per person per day for 100 days per year for highly mechanized agriculture, and 10–100 mg per person per day for both 300 and 150 days per year for labour-intensive agriculture. Exposure for 300 days per year was chosen to represent a landless labourer working for two days per week for each of three employers, and exposure for 150 days per year to represent a person working for three days per week on his or her own land. These exposures represent 'worst case' scenarios as irrigation does not commonly extend over a full year, although in some cases (e.g. coastal desert areas in South America) it does.

For both sub-scenarios we used the following ranges of parameter values in Equations 1 and 2: N, 0.1–1 rotavirus and *Campylobacter* and 0.01–0.1 *Cryptosporidium* oocyst per 10^5 *E. coli* (based on the rotavirus and *Campylobacter* data from waste stabilization ponds in northeast Brazil reported by Oragui *et al.* 1987, and on the *Cryptosporidium* data from

ponds in Kenya reported by Grimason *et al.* 1993), and $\pm 25\%$ of the values given above for ID_{50}, α and r as a preliminary estimate of a reasonable range of values for these parameters. Pathogen numbers in raw wastewater are very variable; the ranges we have used per 10^5 *E. coli* are based on raw wastewater data from tropical countries in non-epidemic situations. However, we recognize that pathogen numbers may be higher (or indeed lower) than the range of values we have used and that therefore the infection risks will be correspondingly higher (or lower). We assumed as worst case situations that there was no pathogen die-off in the soil (thus allowing for the ingestion of freshly irrigated soil particles; moreover oocyst die-off in soil is very slow – Jenkins *et al.* 2002; Nasser *et al.* 2003; Udeh *et al.* 2003); and that the number of *E. coli* per 100 ml of wastewater was also the number of *E coli* per 100 g of soil.

The risks were estimated for two fixed values (10^5 and 10^6 *E. coli* per 100 ml for highly mechanized agriculture, and 10^4 and 10^5 *E. coli* per 100 ml for labour-intensive agriculture), and for seven single-log ranges (10–100 to 10^7–10^8 *E. coli* per 100 ml for both sub-scenarios). These ranges were chosen to estimate the risks associated with different levels of treatment, from untreated wastewater, through treatment to the USEPA & USAID (2004) recommendation of ≤ 200 FC per 100 ml for restricted irrigation, to the State of California (1978) requirement of ≤ 23 total coliforms per 100 ml for restricted irrigation, while allowing for any value to be exceeded by up to one order of magnitude (which could be expected to occur in practice, even if the treatment plant were operating well).

Unrestricted irrigation

We used the same scenario of wastewater-irrigated lettuce consumption (100 g of lettuce per person on alternate days) as used by Shuval *et al.* (1997), but for more pathogen types (*Campylobacter* and *Cryptosporidium*, in addition to rotavirus) and a greater range of wastewater quality. For this scenario the single pathogen dose d in Equations 1–3 is given by:

$$d = [10^m/(100 \times 10^5)] \times V \times N \times 10^{-D}$$

i.e. $d = 10^{m-D-7}VN$ \hfill (4)

where 10^m is the number of *E. coli* per 100 ml of wastewater ($10^m/100$ is the number per ml, and $10^m/(100 \times 10^5)$ is the number of units of 10^5 *E. coli* per ml); *V* is the volume of wastewater remaining on 100 g of lettuce after irrigation, ml; *N* is the number of pathogens per 10^5 *E. coli*; and 10^{-D} is the pathogen die-off between harvest and consumption of the lettuces. Shuval *et al.* (1997) measured *V* as 10.8 ml and used fixed values of 1 and 3 for *N* and *D*, respectively.

We estimated median risks per person per year for rotavirus, *Campylobacter* and *Cryptosporidium* infections resulting from the consumption of 100 g of wastewater-irrigated lettuce on alternate days (i.e. *n* in Equation 3 = 365/2), using the following ranges of parameter values in Equations 1–4: *V*, 10–15 ml (i.e. a narrow range based on the value of 10.8 ml found by Shuval *et al.* 1997); *N*, 0.1–1 for rotavirus and *Campylobacter* and 0.01–0.1 for *Cryptosporidium* oocysts (as for restricted irrigation); *D*, 2–3 for rotavirus and *Campylobacter* (i.e. extending the die-off of 10^{-3} used by Shuval *et al.* 1997 downwards by one order of magnitude) and 0–0.1 for *Cryptosporidium* oocysts (to allow for the slow die-off of oocysts); and $\pm 25\%$ of the values given above for ID_{50}, α and *r*. The risks were estimated for two fixed values (10^3 and 10^4) and eight single-log ranges (1–10 to 10^7–10^8) of *E. coli* numbers per 100 ml of wastewater.

Reference level of acceptable risk

A disease risk of 10^{-3} per person per year (pppy), which is used by WHO (2004) as the tolerable risk of waterborne disease from drinking fully treated drinking water, is unduly cautious (see Haas 1996); it means one occurrence of disease per person per 1,000 years (for a community this risk of 10^{-3} pppy means that every year 0.1% of the community becomes diseased as a result of drinking fully treated drinking water). We consider that a more acceptable level for the risk of infection (rather than of disease) is 10^{-2} pppy (i.e. once in every 100 years, essentially once in a lifetime; or 1% of the community per year). Even this level of risk is relatively cautious, given the much higher actual incidence of diarrhoeal disease occurring in both industrialized and developing countries which is, in order-of-magnitude terms, 0.1–1 pppy (Table 1). Thus a tolerable

Table 1 | Diarrhoeal disease (DD) incidence pppy in 2000 by region and age (Mathers *et al.* 2002)

Region	DD incidence in all ages	DD incidence in 0–4-year-olds	DD incidence in 5–80 + year-olds
Industrialized countries	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

infection risk of 10^{-2} pppy is 1–2 orders of magnitude lower than the average global incidence of diarrhoeal *disease* and so could raise the global average incidence of diarrhoeal disease (even assuming a disease/infection ratio of 1, which is highly unlikely) from 0.7 pppy to only 0.71 pppy. Such a small increase is epidemiologically insignificant (and not easy to measure).

Results

Restricted irrigation

The estimated rotavirus infection risk for highly mechanized agriculture is slightly higher than 10^{-2} pppy for a soil quality of 10^6 *E. coli* per 100 g, but the risks for *Campylobacter* and *Cryptosporidium* are much lower ($\sim 10^{-3}$–10^{-5} pppy) (Table 2). For labour-intensive agriculture the same is true but for a soil quality of 10^5 *E. coli* per 100 g (Tables 3 and 4). Tables 2–4 also show that the infection risks resulting from the use of untreated wastewater (producing 10^7–10^8 *E. coli* per 100 g of soil) are substantial, especially for labour-intensive agriculture: 0.99 pppy for rotavirus and 0.3–0.5 pppy for *Campylobacter*.

Unrestricted irrigation

The estimated rotavirus infection risks, given in Table 5, are $\sim 10^{-2}$ pppy for a wastewater quality of 10^4–10^5 *E. coli* per 100 ml, $\sim 10^{-3}$ pppy for a wastewater quality of 10^3–10^4 *E. coli* per 100 ml, and $\sim 10^{-4}$ pppy for a wastewater quality of 1,000 *E. coli* per 100 ml. This indicates that the current WHO guideline level for unrestricted irrigation may be

Table 2 | Restricted irrigation – highly mechanized agriculture: median infection risks from ingestion of wastewater-contaminated soil estimated by 10,000-trial Monte Carlo simulations*

Soil quality (*E. coli* per 100 g)	Median infection risk pppy		
	Rotavirus	*Campylobacter*	*Cryptosporidium*
$10^7 - 10^8$	0.50	2.1×10^{-2}	4.7×10^{-4}
$10^6 - 10^7$	6.8×10^{-2}	1.9×10^{-3}	4.7×10^{-5}
10^6	2.2×10^{-2}	6.4×10^{-4}	1.5×10^{-5}
$10^5 - 10^6$	6.7×10^{-3}	1.9×10^{-4}	4.6×10^{-6}
10^5	1.5×10^{-3}	4.5×10^{-5}	1.0×10^{-6}
$10^4 - 10^5$	6.5×10^{-4}	2.3×10^{-5}	4.6×10^{-7}
$10^3 - 10^4$	6.8×10^{-5}	2.4×10^{-6}	5.0×10^{-8}
$100 - 1000$	6.3×10^{-6}	2.2×10^{-7}	$\leq 1 \times 10^{-8}$
$10 - 100$	6.9×10^{-7}	2.2×10^{-8}	–

*1–10 mg soil ingested per person per day for 100 days per year; 0.1–1 rotavirus and *Campylobacter,* and 0.01–0.1 *Cryptosporidium* oocyst, per 10^5 *E. coli*; $ID_{50} = 6.7 \pm 25\%$ and $\alpha = 0.253 \pm 25\%$ for rotavirus; $ID_{50} = 896 \pm 25\%$ and $\alpha = 0.145 \pm 25\%$ for *Campylobacter*; $r = 0.0042 \pm 25\%$ for *Cryptosporidium*.

Table 3 | Restricted irrigation – labour-intensive agriculture with exposure for 300 days per year: median infection risks from ingestion of wastewater-contaminated soil estimated by 10,000-trial Monte Carlo simulations*

Soil quality (*E. coli* per 100 g)	Median infection risk pppy		
	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}
10^5	4.3×10^{-2}	1.5×10^{-3}	3.0×10^{-5}
$10^4 - 10^5$	2.0×10^{-2}	7.0×10^{-4}	1.3×10^{-5}
10^4	4.4×10^{-3}	1.4×10^{-4}	3.0×10^{-6}
$10^3 - 10^4$	1.8×10^{-3}	6.1×10^{-5}	1.4×10^{-6}
$100 - 1000$	1.9×10^{-4}	5.6×10^{-6}	1.4×10^{-7}
$10 - 100$	2.0×10^{-5}	5.6×10^{-7}	1.4×10^{-8}

*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*; $ID_{50} = 6.7 \pm 25\%$ and $\alpha = 0.253 \pm 25\%$ for rotavirus; $ID_{50} = 896 \pm 25\%$ and $\alpha = 0.145 \pm 25\%$ for *Campylobacter*; $r = 0.0042 \pm 25\%$ for *Cryptosporidium*.

unduly cautious. Table 5 also shows that requirements for very low levels of faecal indicator bacteria result in corresponding very low levels of risk: the Californian standard of ≤ 2.2 total coliforms (roughly ≤ 1 *E. coli*) per 100 ml (State of California 1978) and the recommendation of USEPA & USAID (1992) for an 'undetectable' level of *E. coli* in 100 ml result in rotavirus infection risks of $\sim 10^{-6} - 10^{-8}$ pppy. Such low levels of risk are difficult to justify epidemiologically, and they are unlikely to be cost-effective in protecting health.

EPIDEMIOLOGICAL EVIDENCE AND COMPARISON WITH ESTIMATED RISKS

Restricted irrigation

Case study 1

Blumenthal *et al.* (2001) reported the data given in Table 6 for the fortnightly prevalence of symptomatic diarrhoeal disease in three field study groups in Mezquital Valley, Mexico, all of which practised restricted irrigation during the dry season. Group 1 used untreated wastewater (6×10^7 FC per 100 ml), Group 2 used partially treated wastewater ($\sim 10^5$ FC per 100 ml), and Group 3 (the control group) practised rain-fed agriculture. The risk of diarrhoeal disease related to wastewater contact was greatest in the last five months of the dry season when people worked in the wastewater-irrigated fields for up to three days per week.

We have extended their data analysis, first, to determine the excess fortnightly prevalence of diarrhoeal disease in those of ≥ 5 years of age in Groups 1 and 2 compared with the control group (0.012 and 0.022 per person per fortnight, respectively, as shown in Table 6); and, second, to calculate the likely excess risks of diarrhoeal disease in these five months by (a) multiplying the reported fortnightly prevalences by 1.3 to correct for likely underestimated respondent recall (Boema *et al.* 1991); (b) interpreting the corrected fortnightly prevalences as 17-day incidences (i.e. 14 days $+3$ days in the previous week when the disease could have

Table 4 | Restricted irrigation – labour-intensive agriculture with exposure for 150 days per year: median infection risks from ingestion of wastewater-contaminated soil estimated by 10,000-trial Monte Carlo simulations[*]

Soil quality (*E. coli* per 100 g)	Rotavirus	*Campylobacter*	*Cryptosporidium*
	Median infection risk pppy		
$10^7 - 10^8$	0.99	0.29	6.6×10^{-3}
$10^6 - 10^7$	0.65	3.1×10^{-2}	6.8×10^{-4}
$10^5 - 10^6$	9.9×10^{-2}	3.2×10^{-3}	7.2×10^{-5}
10^5	2.2×10^{-2}	6.4×10^{-4}	1.5×10^{-5}
$10^4 - 10^5$	9.6×10^{-3}	3.5×10^{-4}	6.8×10^{-6}
10^4	2.3×10^{-3}	6.5×10^{-5}	1.5×10^{-6}
$10^3 - 10^4$	9.6×10^{-4}	2.9×10^{-5}	7.0×10^{-7}
100 – 1000	1.1×10^{-4}	3.0×10^{-6}	7.0×10^{-8}
10 – 100	1.0×10^{-5}	2.9×10^{-7}	7.0×10^{-9}

[*]10–100 mg soil ingested per person per day for 150 days per year; 0.1–1 rotavirus and *Campylobacter*, and 0.01–0.1 *Cryptosporidium* oocyst, per 10^5 *E. coli*; $ID_{50} = 6.7 \pm 25\%$ and $\alpha = 0.253 \pm 25\%$ for rotavirus; $ID_{50} = 896 \pm 25\%$ and $\alpha = 0.145 \pm 25\%$ for *Campylobacter*; $r = 0.0042 \pm 25\%$ for *Cryptosporidium*.

Table 5 | Unrestricted irrigation: median infection risks from the consumption of wastewater-irrigated lettuce estimated by 10,000-trial Monte Carlo simulations[*]

Wastewater quality (*E. coli* per 100 ml)	Rotavirus	*Campylobacter*	*Cryptosporidium*
	Median infection risk pppy		
$10^7 - 10^8$	0.99	0.28	0.50
$10^6 - 10^7$	0.65	6.3×10^{-2}	6.3×10^{-2}
$10^5 - 10^6$	9.7×10^{-2}	2.4×10^{-3}	6.3×10^{-3}
$10^4 - 10^5$	9.6×10^{-3}	2.6×10^{-4}	6.8×10^{-4}
10^4	2.2×10^{-3}	1.3×10^{-4}	4.5×10^{-4}
$10^3 - 10^4$	1.0×10^{-3}	2.6×10^{-5}	3.1×10^{-5}
10^3	2.0×10^{-4}	5.6×10^{-6}	1.4×10^{-5}
100 – 1000	8.6×10^{-5}	3.1×10^{-6}	6.4×10^{-6}
10 – 100	8.0×10^{-6}	3.1×10^{-7}	6.7×10^{-7}
1 – 10	1.0×10^{-6}	3.0×10^{-8}	7.0×10^{-8}

[*]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 oocyst, per 10^5 *E. coli*; $10^{-2} - 10^{-3}$ rotavirus and *Campylobacter* die-off, and 0–0.1 oocyst die-off, between harvest and consumption; $ID_{50} = 6.7 \pm 25\%$ and $\alpha = 0.253 \pm 25\%$ for rotavirus; $ID_{50} = 896 \pm 25\%$ and $\alpha = 0.145 \pm 25\%$ for *Campylobacter*; $r = 0.0042 \pm 25\%$ for *Cryptosporidium*.

started; i.e. the mean duration was assumed to be > 3 days); and (c) using Equation 3 with $P_{I(A)}(d)$ now interpreted as the likely excess of diarrhoeal disease in five months of the dry season and $P_I(d)$ as the corrected excess 17-day diarrhoeal disease incidence, and with $n = [365 \times (5/12) \div 17] \approx 9$. The calculated risks were 0.14 and 0.23 per person per five months for Groups 1 and 2, respectively (Table 6). Thus Group 2, which was exposed to wastewater with fewer FC, had a higher risk; however, the odds ratios for Groups 1 and 2 showed no statistically significant difference (for details see Blumenthal *et al.* 2001); we therefore combined these two groups into one, which thus comprised those with any wastewater exposure, and determined the excess five-month risk for diarrhoeal disease for the combined group (Table 6). The calculated value was 0.18 per person per five months.

Estimated risks. These field risks were simulated by 10,000-trial Monte Carlo runs which were similar to those in Table 3 but for 65 days exposure per year [≈ 3 days per

week for $(52 \times 5/12)$ weeks], a single *E. coli* range of $10^4 - 10^8$ per 100 g of soil and two fixed *E. coli* values of 6×10^7 and 10^5 per 100 g soil (the mean values for Groups 1 and 2, respectively). The estimated median risk for rotavirus infection was 1.4×10^{-2} per person per five months for a wastewater quality of 10^5 *E. coli* per 100 ml (based on our assumption that this was also the quality per 100 g soil) (Table 7). This is one order of magnitude lower than the values of 0.14 and 0.23 per person per five months calculated from the Mexican field data for wastewater qualities of 6×10^7 and 10^5 *E. coli* per 100 ml, respectively (Table 6). The estimated median risks for *Campylobacter* infection for soil qualities of 6×10^7 and $10^4 - 10^8$ *E. coli* per 100 g are close to those calculated from the Mexican field data, but the estimated risk for 10^5 *E. coli* per 100 g was lower by three orders of magnitude.

Table 6 │ Restricted irrigation – Case study 1: fortnightly prevalences and five-month dry-season risks of diarrhoeal disease (DD) in three field study groups in Mezquital Valley, Mexico (Blumenthal *et al.* 2001)

	Group 1 (untreated wastewater: 6×10^7 FC per 100 ml)	Group 2 (partially treated wastewater: $\sim 10^5$ per 100 ml)	Groups 1 and 2 combined	Group 3 (rain-fed agriculture) (control group)
DD + ve/group size	85/1202	104/1282	189/2484	164/2777
Percentage + ve	7.1	8.1	7.6	5.9
Excess percentage + ve	1.2	2.2	1.7	–
Excess DD prevalence per person per fortnight	0.012	0.022	0.017	–
Corrected excess DD prevalence per person per fortnight*	0.016	0.029	0.022	–
Excess DD risk per person per 5 months in the dry season	0.14	0.23	0.18	–

*Factor of 1.3 used to correct for likely respondent underestimate of disease occurrence (Boema *et al.* 1991).

Case study 2

Blumenthal *et al.* (2003) studied the weekly prevalence of symptomatic diarrhoeal disease (defined as ≥ 3 loose stools passed in one 24-hour period) in another field study group in Mezquital Valley, Mexico, during the dry season. This group comprised 2,515 persons of ≥ 5 years of age, of whom 553 were exposed to wastewater containing $\sim 10^4$ FC per 100 ml and used for restricted irrigation, and 1,962 who had no wastewater exposure. Of the former, 42 (7.6%) had symptomatic diarrhoea in a 1-week period and the corresponding figure for the latter was 90 (4.6%), giving an excess percentage diarrhoeal disease prevalence of 3 (i.e. an excess prevalence of 0.03 per person per week). Interpreting this excess weekly prevalence as equivalent to an excess 10-day incidence (i.e. 7 days + 3 days in the previous week when the disease could have started), using Equation 3 with $P_{I(A)}(d)$ interpreted as the likely excess of diarrhoeal disease in five months of the dry season and $P_I(d)$ as the excess 10-day diarrhoeal disease incidence, and with $n = [(5/12) \times (365/10)] \approx 15$, we calculated the likely excess risk of diarrhoeal disease to be 0.37 per person per five months. Thus the exposed group in this case study had a similar risk to the exposed group in case study 1, despite using wastewater of a better quality. The principal reason for this apparent discrepancy was the use of a better epidemiological technique in which (i) for the

measurement of exposure, a measure of individual exposure was used (instead of the exposure of the main farming adult being assigned to all family members); and (ii) an internal control group (where people with contact with wastewater

Table 7 │ Restricted irrigation: 10,000-trial Monte Carlo estimates of infection risks for field conditions in Case Studies 1 and 2 in Mezquital Valley, Mexico*

| Wastewater quality (*E. coli* per 100 ml) | Median infection risk per person per five months | | |
	Rotavirus	*Campylobacter*	*Cryptosporidium*
Case Study 1			
6×10^7	0.99	0.17	3.8×10^{-3}
$10^4 – 10^8$	0.97	0.16	2.7×10^{-3}
10^5	1.4×10^{-2}	4.9×10^{-4}	6.7×10^{-6}
Case Study 2			
10^4	9.1×10^{-2}	3.1×10^{-3}	6.5×10^{-5}
$10^3 – 10^5$	0.33	1.2×10^{-2}	2.5×10^{-4}

*Soil quality per 100 g taken as wastewater quality per 100 ml; 10–100 mg soil ingested per person per day for 65 days in five months; 0.1–1 rotavirus and *Campylobacter*, and 0.01–0.1 *Cryptosporidium* oocyst, per 10^5 *E. coli*; $ID_{50} = 6.7 \pm 25\%$ and $\alpha = 0.253 \pm 25\%$ for rotavirus; $ID_{50} = 896 \pm 25\%$ and $\alpha = 0.145 \pm 25\%$ for *Campylobacter*; $r = 0.0042 \pm 25\%$ for *Cryptosporidium*.

were compared with those living in the same area without such contact) was used instead of an external control group (where the comparison is with people without wastewater contact living in a different area), so reducing the possible effects of confounding factors.

Estimated risks. The results of Monte Carlo risk simulations similar to those done for case study 1 but for wastewater qualities of 10^4 and 10^3–10^5 *E. coli* per 100 ml are given in Table 7. The estimated median rotavirus infection risk of 0.33 per person per five months for a wastewater quality of 10^3–10^5 *E. coli* per 100 ml is very close to the value of the excess diarrhoeal disease incidence of 0.37 per person per five months calculated from the Mexican field data. However, the estimated median risks for *Campylobacter* and *Cryptosporidium* infections are lower by one and three orders of magnitude, respectively.

Unrestricted irrigation

Blumenthal *et al.* (2003) studied the weekly prevalence of symptomatic diarrhoeal disease (defined as ≥ 3 loose stools passed in one 24-hour period) during the dry season in the same communities in Mezquital Valley used in case study 2 above. The study group comprised 1,515 persons of ≥ 15 years of age who ate raw onions which had been irrigated with contaminated river water containing 10^3–10^5 (geometric mean: 3×10^4) FC per 100 ml. Of this number, 381 persons ate onions once per week and were taken, for our present purpose, as the exposed group; 253 ate them less than once per month and thus served as the control group. The percentage of the former who had symptomatic diarrhoea in a 1-week period was 5.5 and in the control group 2.4, giving an excess percentage diarrhoeal disease prevalence of 3.1 (i.e. an excess prevalence of 0.031 per person per week). Using the same assumptions as used in case study 2 above, we calculated the likely excess risk of diarrhoeal disease for frequent consumers of raw onions irrigated with contaminated river water containing a geometric mean FC count of 3×10^4 per 100 ml to be 0.38 per person per five months.

Estimated risks. This field risk was simulated by 10,000-trial Monte Carlo runs similar to those used to derive the

risks in Table 4 but modified to reflect the field conditions by using different ranges of parameter values, as follows

(a) 1–10 and 0.1–1 for *N* for rotavirus and *Campylobacter*, and *Cryptosporidium*, respectively, to allow for the greater number of microorganisms expected to be on the surface of onions than on lettuce (Geldreich & Bordner 1971 found root vegetables irrigated with wastewater containing 5.8×10^4 FC per 100 ml to have an order of magnitude more faecal bacteria than leafy vegetables)

(b) 0.1–1 and 0.01–0.1 for *D* for rotavirus and *Campylobacter*, and *Cryptosporidium*, respectively, to allow for the lower die-off of faecal organisms in soil than on exposed crop surfaces (Strauss 1985)

(c) 1–5 ml per 100 g onions for *V* to allow for a lower volume of wastewater remaining on onions than on lettuce

(d) three fixed values (3×10^4, 1,000 and 100) and one range (10^3–10^5) of *E. coli* per 100 ml of wastewater

For these assumptions and an onion consumption of 100 g per person per week the simulated rotavirus infection risks of 0.39 per person per five months for a wastewater quality of 10^3–10^5 *E. coli* per 100 ml (Table 8) show very close agreement with the measured incidence of diarrhoeal disease of 0.38 per person per five months, but those for *Campylobacter* and *Cryptosporidium* are lower by one and three orders of magnitude, respectively.

Table 8 | Unrestricted irrigation: 10,000-trial Monte Carlo estimates of infection risks for field conditions in Mezquital Valley, Mexico*

Wastewater quality (*E. coli* per 100 ml)	Median infection risk per person per five months		
	Rotavirus	**Campylobacter**	**Cryptosporidium**
10^3–10^5	0.39	1.7×10^{-2}	2.8×10^{-4}
3×10^4	0.29	1.1×10^{-2}	2.3×10^{-4}
1000	1.1×10^{-2}	3.9×10^{-4}	7.6×10^{-6}
100	1.2×10^{-3}	3.2×10^{-5}	8.0×10^{-8}

*100 g of onions consumed per person per week for five months; 1–5 ml wastewater remaining on 100 g onions after irrigation; 1–10 rotavirus and *Campylobacter*, and 0.1–1 *Cryptosporidium* oocyst, per 10^5 *E. coli*; 0.1–1 rotavirus and *Campylobacter* die-off, and 0.01–0.1 oocyst die-off, between harvest and consumption; $ID_{50} = 6.7 \pm 25\%$ and $\alpha = 0.253 \pm 25\%$ for rotavirus; $ID_{50} = 896 \pm 25\%$ and $\alpha = 0.145 \pm 25\%$ for *Campylobacter*; $r = 0.0042 \pm 25\%$ for *Cryptosporidium*.

DISCUSSION

Use of QMRA models

Bradley (1982) noted that 'The real world is infinitely complex. So in dealing with reality the epidemiologist must simplify.' We would extend this to include the risk analyst and to note that neither QMRA nor epidemiology is an exact science and any comparison between the two has to take this into account. Bradley also noted that 'in general simpler models have contributed more to reality than complex ones'. Our Monte Carlo QMRA models are indeed simple (despite being more complex than the single-fixed-values model of Shuval *et al.* 1997): they all use random selections of parameter values within defined linear ranges (as used, for example, by von Sperling 1996 for the design of facultative waste stabilization ponds), rather than selections based on probability density functions. This recognizes the fact that, in most situations in wastewater reuse, and especially in developing countries, there are no data with which to construct such functions. However, if a pathogen has a skewed numerical distribution (which may be expected in wastewaters), the simulated risks may over-estimate or underestimate actual risks, depending on the direction of the skew. However, a start has to be made somewhere, and we believe our approach represents at least a reasonable start.

The infection risks estimated by the 10,000-trial Monte Carlo simulations generally showed relatively good agreement (at least with a difference no greater than one order of magnitude) with disease incidences determined epidemiologically when the assumptions made for the QMRA calculations were close to the conditions in the epidemiological field studies. Thus, for example, the risks in Table 5 (based on the 'theoretical' scenario of lettuce consumption developed by Shuval *et al.* 1997) are not good predictors of the actual disease incidences in Table 6 (arising from onion consumption), whereas those in Table 8 are better predictors as they are based on assumptions much closer to the epidemiological field study conditions. A difference of up to one order of magnitude between epidemiologically determined prevalences of disease and QMRA simulations can be expected since each simulation is for a single pathogen and in the Mexican epidemiological studies no pathogen

identification was done, so the disease measured could have been due to more than one pathogen. This helps explain the lower risks in Tables 7 and 8 for *Campylobacter* and *Cryptosporidium* infections. Since the simulated rotavirus risks were similar to the epidemiologically determined diarrhoeal risks, it is likely that at least one of the causative pathogens was either rotavirus or another virus (or viruses) with a similar infectivity (such as norovirus or astrovirus). This may reflect the fact that the field risks occurred in the dry season, which is also the cool season in Mexico, when viral infections are more prevalent than bacterial infections (which are more common in the rainy/warm season).

Moreover, as noted above, QMRA cannot determine whether an individual becomes infected more than once per year, whereas multiple infections contribute to the epidemiological estimate of disease incidence. Another possible weakness in our simple QMRA model is that infection risks are assumed to rise linearly with changes in water quality, whereas this may not be the case in the field where threshold effects may be in operation (i.e. there may be no epidemiologically detectable disease below a certain wastewater quality, but above this quality disease is detected) (see Table 6). Nor does our model take into account secondary transmission (which adds to the epidemiological total) or protective immunity (which subtracts from it) (Haas & Eisenberg 2001; Eisenberg *et al.* 2004). Finally our model, at least in the way used in this paper, does not consider the variabilities of pathogen concentration which, particularly during epidemics, are likely to be much higher than those used here, with correspondingly higher resultant estimates of infection risks (however, incidence of community disease during an epidemic is unlikely to be wholly due to exposure to wastewater-irrigated crops).

Nevertheless, taking all these points into account, the agreements between our QMRA/Monte Carlo infection risk estimates and the diarrhoeal disease incidences determined epidemiologically are very satisfactory. This provides confidence, when epidemiological data are not available, in the usefulness of QMRA/Monte Carlo models in evaluating the appropriateness of existing standards and guidelines (see Mara 2000; Mara & Horan 2002), and also in developing new standards and guidelines, provided that the assumptions used in the models are close to reality. However, our experience of comparing field-based epidemiological disease rates and QMRA-based infection estimates suggests that it is essential

to obtain at least a minimal quantity of field data before appropriate QMRA models can be constructed; this includes an estimate of variations in wastewater quality over the exposure period considered, knowledge of the type(s) and duration of exposure (e.g. restricted or unrestricted irrigation; for the former whether the exposure is via highly mechanized or labour-intensive agriculture, and for the latter a knowledge of the type(s) of vegetable(s) consumed and the frequency of consumption). Indeed more than one QMRA model may be needed: for example, in the case of unrestricted irrigation, one for each of the different vegetables, or types of vegetable (root and non-root crops) consumed.

Recommendations for revising the WHO guidelines

We consider the implications of the available evidence, including the QMRA results obtained above, for modifying the WHO guidelines for wastewater use in both restricted and unrestricted crop irrigation based on an acceptable risk of 10^{-2} pppy and taking into account the current community incidences of diarrhoeal disease (Table 1).

Restricted irrigation

(a) *Labour-intensive agriculture*. The estimated risks for rotavirus infection are slightly higher than 10^{-2} pppy for a wastewater quality of 10^5 *E. coli* per 100 ml, but for *Campylobacter* and *Cryptosporidium* they are lower by 1–3 orders of magnitude (Tables 3 and 4). However, epidemiological evidence from Mexico (Blumenthal *et al.* 2003) shows that children under the age of 15 are not always protected by $\leq 10^5$ *E. coli* per 100 ml; $\leq 10^4$ *E. coli* per 100 ml does protect them. Thus a guideline value of $\leq 10^5$ *E. coli* per 100 ml is appropriate subject to two provisos: (i) that human exposure techniques (Blumenthal *et al.* 1989; WHO 1989) are used as additional protective measures for fieldworkers in developing countries, and (ii) that when children under the age of 15 are exposed the guideline value is reduced to $\leq 10^4$ *E. coli* per 100 ml.

(b) *Highly mechanized agriculture*. The estimated risks for rotavirus infection are slightly higher than 10^{-2} pppy for a wastewater quality of 10^6 *E. coli* per 100 ml, but for *Campylobacter* and *Cryptosporidium* they are lower by

2–3 orders of magnitude (Table 2). This suggests that the guideline value of $\leq 10^5$ *E. coli* per 100 ml could be safely relaxed in this case to $\leq 10^6$ *E. coli* per 100 ml.

Unrestricted irrigation

(a) *Root crops*. The estimated risks for rotavirus infection, based on the epidemiological data for raw onion consumption in Mexico, are $\sim 10^{-2}$ pppy for a wastewater quality of 1,000 *E. coli* per 100 ml (Table 8), thus confirming the appropriateness of the current WHO guideline value of $\leq 1,000$ *E. coli* per 100 ml.

(b) *Non-root crops*. The estimated risks for rotavirus infection from lettuce consumption are $\sim 10^{-2}$ pppy for a wastewater quality of 10^4–10^5 *E. coli* per 100 ml (Table 5), which suggests that the current WHO guideline value of $\leq 1,000$ *E. coli* per 100 ml can be safely relaxed to $\leq 10^4$ *E. coli* per 100 ml when root crops are not grown. Support for this relaxation is provided by the guideline value for ready-to-eat foods in England and Wales (Gilbert *et al.* 2000), which is used in many other countries, including Australia, Canada and New Zealand (Institute of Medicine 2003): these are considered to be of 'acceptable' quality if they contain < 100 *E. coli* per gram wet weight (i.e. $< 10^4$ per 100 g) (Gilbert *et al.* 2000). Since lettuce is a common component of many ready-to-eat foods, it makes little sense for the wastewater used to irrigate lettuce to be treated to a higher quality than is required of the lettuce itself.

CONCLUSIONS

- The combination of standard QMRA techniques and multi-trial Monte Carlo risk simulations is a useful technique for evaluating microbiological quality guidelines and standards for treated wastewaters used for both restricted and unrestricted irrigation against any level of acceptable risk.
- To achieve an acceptable risk of 10^{-2} pppy for rotavirus infection the model scenario of involuntary soil ingestion for restricted irrigation indicates that the required wastewater quality should be $\leq 10^5$ *E. coli* per 100 ml in the case of labour-intensive agriculture, and $\leq 10^6$ *E. coli* per 100 ml in the case of highly mechanized

agriculture. However, human exposure techniques should be additionally employed for labour-intensive agriculture and the required wastewater quality should be reduced to $\leq 10^4$ *E. coli* per 100 ml when children under the age of 15 are exposed. For the same level of acceptable risk the model scenario of lettuce consumption for unrestricted irrigation indicates that the current WHO guideline level of $\leq 1,000$ *E. coli* per 100 ml can be safely relaxed to $\leq 10^4$ *E. coli* per 100 ml when root crops eaten uncooked are not grown; however, when they are grown the current guideline value of $\leq 1,000$ *E. coli* per 100 ml is required.

- Comparison between the diarrhoeal disease incidences determined in epidemiological field studies and QMRA/-Monte Carlo-simulated risks of infection are only meaningful if the parameter values used in the risk assessment are close to those likely to occur in the field. Using appropriate parameter values good agreement was obtained between simulated risks and those determined in recent epidemiological field studies in Mexico.

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