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Zero Risk Does Not Exist: Lessons Learned from Microbial Risk Assessment Related to Use of Water and Safety of Fresh Produce

Ann De Keuckelaere, Liesbeth Jacxsens, Philip Amoah, Gertjan Medema, Peter McClure, Lee-Ann Jaykus, and Mieke Uyttendaele

Abstract: Risk assessments related to use of water and safety of fresh produce originate from both water and food microbiology studies. Although the set-up and methodology of risk assessment in these 2 disciplines may differ, analysis of the current literature reveals some common outcomes. Most of these studies from the water perspective focus on enteric virus risks, largely because of their anticipated high concentrations in untreated wastewater and their resistance to common wastewater treatments. Risk assessment studies from the food perspective, instead, focus mainly on bacterial pathogens such as *Salmonella* and pathogenic *Escherichia coli*. Few site-specific data points were available for most of these microbial risk assessments, meaning that many assumptions were necessary, which are repeated in many studies. Specific parameters lacking hard data included rates of pathogen transfer from irrigation water to crops, pathogen penetration, and survival in or on food crops. Data on these factors have been investigated over the last decade and this should improve the reliability of future microbial risk estimates. However, the sheer number of different foodstuffs and pathogens, combined with water sources and irrigation practices, means that developing risk models that can span the breadth of fresh produce safety will be a considerable challenge. The new approach using microbial risk assessment is objective and evidence-based and leads to more flexibility and enables more tailored risk management practices and guidelines. Drawbacks are, however, capacity and knowledge to perform the microbial risk assessment and the need for data and preferably data of the specific region.

Keywords: fruits and vegetables, health risk, mitigation strategies, quantitative microbial risk assessment, water

Practical Application: This manuscripts intends to give an extensive overview of approaches and challenges of past and future quantitative microbial risk assessment studies in the fresh produce chain related to the use of water in order to aid further research efforts in this area.

Introduction

Foodborne illnesses may originate from poor water quality used in fresh produce production. Fecal contaminated irrigation water has been implicated as either a possible source, or a likely source of pathogen contamination of fresh, raw consumed fruits and vegetables (for example, Thurston-Enriquez and others 2002; Okafo and others 2003; Ensink and others 2007; Leifert and others 2008). Water used for irrigation may originate from multiple sources and include rain water, ground water, surface water, (treated) wastewater, or even desalinated seawater. The availability of water sources for irrigation is under increasing pressure. Reconditioned waste or surface water are 2 abundant sources with potential to replace untreated ground or rain water. Application of alternative water sources may result in an elevated probability of the presence of pathogens and may increase the pressure on governing water quality (WHO 2006). Guidelines or even criteria on the quality of water applied for irrigation in fresh produce production are set by some countries or individual states such as Canada (Steele and Odumeru 2004) and Spain (Iglesias and others 2004). Most guidelines are empirically derived fixed microbial standards focusing on defined indicator organisms or pathogens. Risk assessment strategies to underpin management of health risks are evidence-based and may also be helpful and provide flexibility in setting guidelines for specific situations. In recognition of this, WHO has replaced the original approach of water quality testing for fecal coliforms to evaluate compliance with a guideline of <1000 fecal coliforms per 100 mL (Blumenthal and Peasey 2002) by a risk assessment/risk management-based approach with

MS 20142009 Submitted 8/12/2014, Accepted 18/3/2015. *Authors De Keuckelaere, Jacxsens, and Uyttendaele are with Dept. of Food Safety & Food Quality, Faculty of Bio-Science Engineering, Ghent Univ., Belgium. Author Amoah is with Intl. Water Management Inst. (IWMI), Accra, Ghana. Author Medema is with Water Quality & Health, KWR Watercycle Research Inst. and Water Management, Faculty of Civil Engineering & Geosciences, Delft Univ. of Technology, the Netherlands. Author McClure is with Mondelez Intl., Bayerwaldstrasse 8, München, Germany. Author Jaykus is with North Carolina State Univ., N.C., U.S.A. Direct inquiries to author Jacxsens (E-mail: liesbeth.jacxsens@ugent.be).*

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more flexible guidelines based on attributable risks and disability-adjusted life years in the WHO guidelines for use of wastewater in agriculture (WHO 2006). These guidelines provide the framework for national and local decision making to manage the health risk from hazards associated with (treated) wastewater or other alternative sources of water use in agriculture. A similar approach was used in establishing Australian guidelines for water recycling (NRMMC-EPHC-AHMC 2006; O'Toole and others 2010).

Aside from irrigation water, washing of produce at harvest, during further processing, or during preparation may also function as a means of foodborne pathogen contamination of produce (Gil and others 2009; Holvoet and others 2014). In postharvest practice, both the prevention of cross-contamination during the washing process by applying sanitizers, and the reconditioning of spent water for subsequent reuse, have been extensively studied (Lopez-Galvez and others 2009; Van Haute and others 2013, b; Luo and others 2014). However, if occasional contamination does occur, even with adequately operated and monitored washing procedures, microbial concentrations are reduced by only 1 to 2 log units at best (Beuchat 1998). The inclusion of a washing step can therefore result in an increase or decrease on the occurrence of contaminated crops or fresh-cut produce, but its efficacy will depend upon initial pathogen load as well as the ability to maintain the washing water quality being used during processing and preparation.

The selection of water source, water treatment, and water quality for use throughout the fresh produce supply chain must consider a wide variety of crops, production practices, and consumption patterns. Therefore, a flexible approach must be applied in setting microbiological guidelines or criteria for types and uses of water.

The principal aim of microbial risk assessment (MRA) is to support risk management by providing an objective, transparent, evidence-based assessment of the health risk of (different) exposure pathways/scenarios. In the case of water use in fresh produce primary production, risk assessment crosses 2 scientific disciplines, those being environmental (water) science and food science. Although epidemiological studies (observing exposed and non-exposed populations) may also be used to assess risk, and some of these have been carried out to assess risks associated with drinking water, they are costly and the logistics, limited sensitivity in measuring disease, and specific populations being studied mean that quantitative MRAs are often preferred.

The first MRAs for water use in fresh produce production were initiated by risk assessors with a "water" background that investigated the contaminated fresh produce as one possible exposure pathway for microbial contaminated reclaimed water (for example, in Asano and others 1992; Tanaka and others 1998). The focus of these initial MRA studies was treatment of waste water and irrigation practices at the farm level. Gradually studies became available that focused on other parts of the fresh produce supply chain, including washing and cutting of fresh produce (for example, Carrasco and others 2010; Rodriguez and others 2011) and integrating the role of consumer preparation (for example, Domenech and others 2013). These latter MRAs were executed by risk assessors with a background in food science. As both scientific disciplines have a different perspective and developed their own approach toward MRA, cross-pollination between different disciplines is recommended to expand expertise and promote collaborative understanding (O'Toole and others 2014). The aim of the present study is to review environmental and food science MRA studies on water and safety of fresh produce to develop a holistic assessment from source water in the farm-to-fork chain where water is included as a potential vehicle for foodborne pathogens.

During the review specific consideration is given to production (for example, irrigation water) or microbial removal strategies (such as washing). The selected quantitative MRAs (QMRA) were further analyzed in-depth to: (1) obtain insights in overall approaches used during QMRA modeling; (2) identify investigated mitigation strategies by scenario analysis; (3) summarize assumptions made and any surrogate data; (4) identify recurring data gaps; and (5) characterize how risk is expressed and, if applicable, compared with acceptable levels of protection targets. Finally, lessons learned and recommendations for future risk assessment studies are made.

Materials and Methods

Screening of peer-reviewed literature and collection of QMRA publications

Relevant publications from peer-reviewed literature were selected on the basis of the following criteria: (i) a quantitative risk assessment or exposure model calculating the likelihood of infection, illness or presence of (ii) a defined microbial foodborne pathogen (bacteria, viruses, protozoa, or helminths) (iii) through the consumption of fresh produce or occurrence on fresh produce, and (iv) which included the modeling of effect of water use or water treatment on the quality of fresh produce in at least one stage of the farm-to-fork supply chain. Water could have a role in the transmission of the foodborne pathogen to fresh produce during irrigation with contaminated water or during the postharvest washing process (in fresh-cut processing) or salad preparation. QMRA articles were identified by searching Thomson Reuters Web of Science™ (formerly ISI Web of Knowledge) Core collection and further search by screening the reference list of identified relevant QMRA articles. Studies that were not selected included one in which a QMRA was done based on the use of urine as irrigation water (Hoglund and others 2002), and one that failed to give specific detail on how the risk calculation was performed (Aiello and others 2013). Other QMRAs dealing with the safety of fresh produce, but that did not include water in any step of the model (Franz and others 2010; Verhaelen and others 2013), were also excluded. The collection of publications ended in December 2013.

Classification of selected QMRA publications

In total, 41 QMRA studies were selected (Table 1). Studies were classified according to the target pathogen(s) under investigation, which could be either a foodborne virus, parasitic protozoon, bacterium, or helminth. A subclassification was made to describe in which part of the farm-to-fork continuum (production, packing/processing including distribution, consumer home) the impact of water was considered. In case irrigation water was included, it was noted if the study also included the effect of prior water treatment on irrigation water quality. Studies were also subclassified according to the background/perspective of the risk assessment team. This was done by searching in the affiliations of the authors with the terms "water," "environmental," "food," and "agriculture." When "water" or "environmental" was present for one or more of the name(s), the article was classified as written from a "water perspective." When "food" or "agriculture" was present, the article was classified as written from a "food perspective." When the author names included terms from both groups, the article was classified as written from a "water and food perspective."

Table 1–Classification (in chronological order) of the selected QMRA studies according to the target pathogen, part in the farm-to-fork chain in which the effect of water was included, background of the involved research groups, and type of QMRA.

RA studies	Norovirus	Rotavirus	Hepatitis A virus	Enterovirus	Enteric virus	*Giardia* spp.	*Cryptosporidium* spp.	*Entamoeba histolytica*	*Campylobacter*	*L. monocytogenes*	Pathogenic *E. coli*	*Salmonella*	Enteric pathogenic bacteria	Ascaris	Water treatment	Irrigation	Washing$_{Processing}$	Washing$_{consumer}$	Water-perspective	Food-perspective	Deterministic RA	Stochastic RA
Asano and others (1992)					X										X	X			X		X	
Shuval and others (1997)		X	X													X		X*	X		X	
Tanaka and others (1998)					X										X	X			X			X
van Ginneken and Oron (2000)					X										X	X			X			X
Petterson and Ashbolt (2001)					X											X			X			X
Petterson and others (2001a); Petterson and others (2002)				X												X			X			X
Stine and others (2005)			X									X				X			X	X	X	
Hamilton and others (2006a)					X											X			X			X
Hamilton and others (2006b)					X											X			X			X
NRMMC-EPHC-AHMC (2006); O'Toole and others (2010)		X					X		X							X			X		X	
Mara and others (2007)		X					X		X							X			X			X
Bastos and others (2008)		X				X	X		X							X			X		X	
Diallo and others (2008)						X	X				X					X			X			X
Seidu and others (2008)		X												X		X	X		X			X
Finley and others (2009)													X			X			X		X	
Mota and others (2009)						X	X									X				X	X	
Navarro and others (2009)														X				X	X		X	
Al-Juaidi and others (2010)					X											X			X			X
Barker-Reid and others (2010)					X											X[c]			X			X
Carrasco and others (2010)										X							X			X		X
Forslund and others (2010)		X														X			X	X		X
Mara and Sleigh (2010a)														X		X			X			X
Mara and Sleigh (2010b)	X															X			X			X
Munoz and others (2010)				X												X			X		X	
Oron and others (2010)		X													X	X			X			X
Ayuso-Gabella and others (2011)		X					X		X						X	X		X	X			X
Danyluk and Schaffner (2011)											X						X			X		X
Drechsel and Seidu (2011)		X					X					X			X	X	X		X			X
Navarro and Jimenez (2011)														X		X		X	X		X	
Ottoson and others (2011)											X					X		X		X		X
Rodriguez and others (2011)											X						X			X		X
Stine and others (2011)			X									X				X[a]			X	X	X	
Ferrer and others (2012)						X		X								X			X			X
Forslund and others (2012)		X														X			X	X		X
Barker and others (2013)	X															X[c]		X	X	X		X
Ding and others (2013)										X								X		X		X
Domenech and others (2013)										X								X		X		X
Lim and Jiang (2013)						X						X				X[c]			X			X
Pavione and others (2013)		X					X		X							X		X[d]	X			X
Puerta-Gomez and others (2013)												X					X			X		X
Seidu and others (2013)											X			X		X			X		X	

[a] Not the effect of contaminated irrigation water but effect of contaminated pesticide spray water was investigated on contamination level of fresh produce.

[b] Under pathogen category "pathogenic *E. coli*" in all cases but one *E. coli* O157 was the target organism, only in Diallo and others (2008) the pathogenic *E. coli* under study was "diarrhea causing *E. coli*."

[a] The QMRA is undertaken partially (as a scenario) for home-produced vegetables (Barker-Reid and others (2010)) or performed only for home-produced vegetables (Barker and others (2013) and Lim and Jiang (2013)).

[d] Indicates that the removal effect of the washing was included in a joint removal factor with postharvest survival.

A final subclassification for the studies was made based on the type of QMRA that was performed: deterministic or stochastic risk assessment.

In-depth analysis of selected QMRA publications

The selected QMRAs were analyzed in-depth to summarize the overall approaches taken in modeling, and the use of assumptions and surrogate data in an effort to identify recurring data gaps. Such data gaps, assumptions, and surrogate data dealt with issues such as: (1) pathogen contamination prevalence data; (2) transfer rates for pathogens from water to produce; (3) behavior (growth, survival, inactivation, removal) of microorganisms in the environment and produce; (4) consumer behavior and consumption patterns; and (5) dose–response information. The diversity in risk end-point and characterization/benchmarking, such as the use of a tolerable or acceptable risk level, are also identified and discussed.

Results on the outcomes of the QMRA studies are discussed in a second part of this review. This includes the major lessons learned about the intervention strategies or control measures that were investigated relative to water use in the fresh produce supply chain. Also future perspectives in risk assessment related to water and the safety of fresh produce are discussed.

Results and Discussion

Food science versus water microbiology: a different perspective

In total, 41 QMRA studies were identified for further characterization. The majority of these contained at least one stochastic variable (29/41), only 12 models were deterministic. Most QMRA studies were elaborated by a "Water/Environmental-group" (Water perspective) (28/41), 8 studies were conducted from a "Food/Agriculture-group" (Food perspective), and 5 studies were done by combined research groups representing both Water Microbiology and Food Science. This is not surprising as risk assessment for water safety or wastewater reclamation has a much longer tradition compared to risk assessment for the food sector. Earlier QMRA studies considered consumption of food crops only as one of several possible exposure scenarios of (treated) wastewater, and the effect and efficiency of wastewater treatment was the main objective of those studies (as in Asano and others 1992; Tanaka and others 1998; van Ginneken and Oron 2000).

Variation in focus on the stage in the farm-to-fork model under consideration. For those studies written from a Water perspective (28/41), all included the Farm level as a part of the "farm-to-fork model"; 6 studies included Wastewater treatment, 6 included the Consumer level, and only 2 included the processing level of fresh-cut produce, although the processing step was not an industrial process but a washing step conducted by street food venders (Seidu and others 2008; Drechsel and Seidu 2011). Studies published by research groups with a Food perspective date back to only 2005 (Stine and others 2005). In the farm-to-fork continuum, most of these studies included Farm level (8/13), but compared to QMRAs performed by research groups with a Water background, the role of Packing/Processing (5/13) and the Consumer (8/13) were more often incorporated and water treatment was not included (0/13).

Variation in focus of target pathogen under consideration. The papers dealt with a wide range of pathogenic bacteria (such as *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and *Campylobacter* spp.), viruses (human enteric viruses, enterovirus, hepatitis A virus, norovirus, and rotavirus), parasitic protozoa (*Cryptosporidium* spp., *Giardia* spp., and *Entamoeba histolytica*), and helminths (*Ascaris lumbricoides*). Articles written from a Water perspective tended to focus on human enteric viruses (20/28). It appeared that the choice of these pathogens was not driven by data availability but rather because enteric viruses are known to be highly infective, are often found in high concentrations in secondary effluent, are relatively persistent in the environment, and are believed to be responsible for the majority of waterborne infections in developed countries such as the United States (Hamilton and others 2006a, b). Five of these QMRA studies elaborated from the Water perspective use so-called "reference pathogens." These are selected pathogens, the control of which is stringent enough to be considered adequate to control other pathogen classifications to a similar or greater extent. This concept was introduced by the World Health Organization (WHO) to aid in setting guidelines for wastewater reuse and water treatment requirements (WHO 2004; Gibney and others 2013). Typical reference pathogens are *Campylobacter* spp. (or *Salmonella* spp.; Drechsel and Seidu 2011), rotaviruses (Ayuso-Gabella and others 2011), and *Cryptosporidium* to control for risks related to bacteria, viruses, and parasitic protozoa and helminths, respectively. The reference pathogen concept is debatable and may also be a source of confusion. QMRA studies should probably justify the choice of reference micro-organism(s), particularly by specifying if the reference is considered the most hazardous organism of concern or is merely used because better data are available for that organism (for example, in terms of prevalence data or dose–response relationships). For example, in assessing the viral risk for setting the Australian guidelines for water recycling (NRMMC-EPHC-AHMC 2006), rotavirus is used as a reference pathogen in risk assessment calculations. However, adenovirus data were used in place of rotavirus data for wastewater because there is a lack of data for rotavirus. Still the relevant DALY information for rotavirus was included in the modeling as rotavirus was the reference.

When QMRAs were performed for different classes of pathogens (viruses, bacteria, protozoa), the viruses most commonly presented the highest risk of infection (Mara and others 2007; Bastos and others 2008; Pavione and others 2013). All 5 QMRA studies focusing on *A. lumbricoides* were also performed from a Water perspective. Studies from a Food or combined Food and Water perspective mostly focused on specific enteric foodborne pathogens such as *E. coli* O157:H7 ($n = 3/13$), *Salmonella* ($n = 3/13$), and *L. monocytogenes* ($n = 3/13$). Although also viruses ($n = 5/13$) such as hepatitis A, norovirus, and rotavirus, and parasitic protozoa ($n = 1/13$) (*Cryptosporidium* and *Giardia*) were included as target pathogens.

Variation in focus on food crop under consideration. More than half of the 41 publications were QMRA studies concerning leafy vegetables such as salad crops, lettuce (for salads), or spinach. Other commodities included bell peppers, cucumber, broccoli, cabbage, onion, kale, carrots, tomatoes, potato, and cantaloupe. Leafy greens are prone to contamination with pathogens as they have large surface area (hence, greater pathogen attachment sites), are grown in close proximity to the soil, irrigated intensively, and are mainly consumed raw (Melloul and others 2001; Vega and others 2005). Among fresh fruits and vegetables, leafy green vegetables and fresh herbs were perceived as of greatest concern in terms of microbiological hazards and received the highest priority in a joint expert meeting of FAO and WHO (FAO/WHO 2008). This study and others (such as EFSA BIOHAZ Panel 2012; Chen and others 2013) are based on qualitative ranking of certain parameters by experts, are no QMRA studies and are, therefore, not used in this

review. However, they can be of interest for risk managers to set priorities in certain pathogen/commodity combinations.

Modeling strategies, use of assumption and surrogate data, recurring data gaps

Quantitative risk assessment studies have to be fit for intended purpose and demand a combination of data collection, mathematical modelling or calculations, and expert insights and interpretations. Depending on the required objective and nature of available information, each assessment will result in a particular strategy or approach. So, although there is guidance on good QMRA practice, it is impossible to set a "gold standard" for these types of studies (CAC 1999, 2007a, b). Risk assessments are data intensive and require data on a specific (usually national) context. Risk assessments are often confronted with variability or uncertainty in data sets (Vasquez and others 2014). Variability in data is because of intrinsic variance and cannot be reduced by increasing sample size, it is inherent to living or real-world systems (such as prevalence of pathogens in water or consumption habits of populations). Lack of data can lead to the inclusion of uncertainties in the QMRA calculations. Uncertainty can be reduced by additional data collection; however, this is often in practice not feasible (such as in case of dose–response modelling). Thus, risk assessors are often confronted with lack of information and need to use surrogate data or assumptions. In the frame of the present manuscript, the term "assumption" is further defined, according to the Oxford Dictionary, as information which is accepted as true, *without* (experimental) proof for the specific setting. Assumptions are frequently based on expert opinion and may well lack consideration of variability. The term "surrogate data" is used when stand-in or substituted data are based on (limited) experiments or when data obtained for another microorganism or situation is used as a proxy for the pathogen or situation under study. Examples of surrogate data are the use of data of another microorganism than that of concern, or another country than that of interest. In the absence of high-quality data, use of surrogate data or assumptions often leads to more conservative estimates, also referred to as worst case scenarios. Constraints, uncertainties, and assumptions having an impact on the risk assessment should be explicitly considered and documented in a transparent manner (CAC 2007b).

By detailed analysis of the selected QMRA studies, surrogate data or assumptions were identified for each of the following data categories: (1) prevalence and concentration of microorganisms of concern; (2) transmission routes (how the pathogens enter the food chain); (3) growth, removal, survival, and/or inactivation of microorganisms; (4) consumer behavior; and (5) dose–response relationship.

Filling the data gap on prevalence and concentration of pathogens in water or fresh produce along the fresh produce chain. To assess exposure, the prevalence and concentration of pathogens on the commodity under consideration—or further backwards in the supply chain (such as in irrigation water)—needs to be known. This was one of the major data gaps identified during this review. There are many approaches taken to overcome this data gap problem, as listed in Table 2.

For many reasons, there is little routine or regular monitoring of fresh produce or water for the presence of pathogens in most countries, explaining the lack of data. Even when done, pathogen prevalence is usually quite low. For example, *Salmonella* spp. prevalence reported in foods of nonanimal origin as part of the European Food Safety Authority's (EFSA's) zoonoses web-based reporting from 2004 to 2011 was 0.48%. In another example, of 1860 samples of unprocessed leafy raw vegetables (from October 2006 to October 2007) sampled at the entrance hall of 2 processing companies in The Netherlands, *Salmonella* spp. were detected in 6 samples (0.38% prevalence estimate) in the range of 0.019 to 0.281 CFU per gram (Pielaat and others 2014). Clearly, obtaining accurate data requires large sample numbers in order to construct an adequate probability distribution of pathogen concentration/prevalence for the model. Even if a large data set was collected, the sample volume and location is an important factor to be taken into account. Samples could be falsely reported as negative because of pathogen concentrations falling below assay detection limits. Indeed, factors such as assay specificity, sensitivity, and availability of internationally standardized analytical methods all affect the quality of the data obtained. In case of detection by molecular methods (such as for viruses), a positive test result obtained by real-time PCR does not mean that the pathogen is infectious and thus a public health hazard (Knight and others 2013). Some studies assume that genomic copies detected can be equated to numbers of infective pathogens (Barker and others 2013; Ferrer and others 2012; Lim and Jiang 2013), but this may overestimate public health risk.

To take into account the impact of positive samples having pathogen concentrations below assay detection limits, additional steps in dealing with sampling data may be imposed in some QMRAs (such as by Ding and others 2013; Lim and Jiang 2013). Mota and others (2009) applied a deterministic approach by simply performing the calculation of annual risk of infection using the limit of detection of the method involved. To consider seasonal fluctuations of pathogen loads (as in reclaimed wastewater used for irrigation), it was important to sample for a prolonged period of time, comprising the whole crop cycle and growing season (Diallo and others 2008). As a result of temperature and rainfall variations, and overflows or occasional household or industrial discharges, spatial and temporal variability are typically observed in microbial parameters of surface water (Nnane and others 2011; Won and others 2013). In this literature review, different approaches were identified to handle data needs related to the prevalence and concentration of microorganisms in water or fresh produce (Table 2).

The *first approach* and preferred situation occurs when QMRA studies have access to relevant sampling and pathogen testing data, either data through collection by the team doing the modeling (as by Ferrer and others 2012) or through pathogen data availability from prior representative studies, such as same region, same type of water, or food crop under consideration (Ding and others 2013). It is important that the suitability and robustness of the data set being used as input into QMRA is verified relative to sampling plan (number of samples and sampling locations) and analytical method performance (specificity, sensitivity, and limit of detection). If situation-specific data on pathogen presence (for example, [reclaimed] irrigation [waste]water or on produce) are missing, other strategies are used to obtain plausible estimates. As such, a *second approach* is the use of data from other production sites/countries (as by Lim and Jiang 2013) or from other (similar) vegetables (as by Carrasco and others 2010) as surrogates for the situation under study.

A *third approach* is the use of established ratios between indicator bacteria and the specific pathogen under consideration. This strategy is used to circumvent the problem of analyzing large sample numbers or sizes for the presence of specific pathogens of low prevalence, as data for indicators are more readily available, of higher prevalence, and higher concentration. Some of the

Table 2–Data needs related to prevalence and concentration of pathogens in water or fresh produce and identified approaches (based on genuine data, surrogate [S] data or assumptions [A]) including their inherent frailty to deal with these data needs.

Data need on pathogen concentration and prevalence (including seasonal fluctuations) on fresh produce and/or in (reclaimed) water used for irrigation practices.	
(i) The use of relevant prevalence and concentration data obtained by own sampling or from prior studies being representative (same region, same type of water or food crop under consideration)	Ding and others (2013); Ferrer and others (2012)
(ii) The use of data from another production site/countries or other similar vegetables as **surrogate** data	Barker-Reid and others (2010); Carrasco and others (2010); Lim and Jiang (2013)
(iii) The use of **ratios between indicator bacteria and the specific pathogen** under study, based on limited experimental data, to estimate the pathogen concentration level in the initial irrigation water in a different context. For example,	
– Ratio enteric virus : fecal coliform in wastewater is 1:10^5	Munoz and others (2010); Shuval and others (1997)
– 0.1–1 rotavirus per 10^5 *E. coli* (or fecal coliform)	Mara and others (2007); Pavione and others (2013)
– 0.1–1 *Campylobacter* per 10^5 *E. coli*	Bastos and others (2008); Mara and others (2007)
– 0.01–0.1 *Cryptosporidium* (oocyst) per 10^5 *E. coli*	Mara and others (2007); Pavione and others (2013)
– Others	
When using these ratios in a different context than those observed during the experimental studies on which these are based, some **assumptions** are made:	Mara and Sleigh (2010b); Seidu and others (2008)
• The contributing source of fecal load (human/non-human) to the water is similar as in the experimental study	
• The removal efficiency of the used WWT or the survival and growth of the indicator and the pathogen are comparable. • There is a linear relationship between the concentration of the indicator and the concentration of the pathogen of interest In order to use the ratios with the data that was at hand some indicators/microorganisms were used as **assumed surrogate** for others, for example, – *E. coli* accounts for all fecal coliforms enteric virus : *E. coli* ratio is 1 : 10^5 – Enteric viruses are represented by enteroviruses – Data of total coliforms was used instead of fecal coliforms – NoV are represented by enteroviruses = > 0.1 – 1 norovirus per 10^5 *E. coli* Ratios (pathogen/indicator ratios) that were initially based on occurrence in (treated) municipal wastewater were **assumed** to be applicable to calculate number of pathogens present ON produce (for example, tomatoes, potatoes, lettuce) For other pathogen/indicator ratios there is no clear reference to experimental/screening studies, for example, 8% of measured *E. coli* concentration is diarrhegenic/pathogenic or a pathogen/indicator ratio was assumed: for example, ratio of *E. coli* O157:H7 to *E. coli* is 1:10^6.	Barker-Reid and others (2010); Drechsel and Seidu (2011); Munoz and others (2010) Mara and others (2007); Mara and Sleigh (2010a); Mara and Sleigh (2010b) Barker-Reid and others (2010) Munoz and others (2010) Mara and Sleigh (2010b) Forslund and others (2010); Forslund and others (2012); Seidu and others (2008)
(iv) The fecal loading approach to estimate the concentration of a specific pathogen in water.	Diallo and others (2008) Seidu and others (2013)
(v) The initial contamination level of the (initially untreated) irrigation water or produce was simulated by different potential contamination levels, or the use of one assumed scenario.	Barker and others (2013); Ottoson and others (2011) Bastos and others (2008); Domenech and others (2013); Mara and others (2007); Rodriguez and others (2011); Or Oron and others (2010); Van Ginneken and Oron (2000)

ratios most often used are described in Table 2. Almost all studies that use one of these ratios for estimating the concentration of enteric viruses, *Campylobacter* or *Cryptosporidium*, refer to only 2 limited experimental studies. The ratios for rotavirus, enterovirus, and *Campylobacter* spp. are based on data from waste stabilization ponds in northeast Brazil reported by Oragui and others (1987), the ratio for *Cryptosporidium* is based on data from ponds in Kenya reported by Grimason and others (1993). Both studies determined the number of fecal coliforms together with the concentration of these pathogens. The widespread application of these ratios to situations very different from those encountered in the initial data collection, such as raw wastewater data from tropical countries in nonepidemic situations by Mara and others (2007), is not supported experimentally. For example, the diversity of pathogens present and concentrations in raw sewage depend upon origin of the fecal input (human/nonhuman sewage; O'Toole and others 2014) and the epidemiological status of the contributing populations (Hamilton and others 2007), both of which differ by region. This is particularly relevant when applying these ratios to QMRA for use in developed countries (Forslund and others 2010) or epidemic situations. The use of these ratios as proxy for other types of water such as (partially) treated wastewater (Munoz and others 2010) or domestic greywater (Barker-Reid and others 2010) is also questionable because of differences in wastewater treatment efficiency and the comparability of survival/growth of indicators versus pathogens.

The case for enteric viruses is a good one. For example, there is ample evidence that enteric viruses may persist after water disinfection treatments that eliminate bacteria (Ottoson and others 2006; Simmons and Xagoraraki 2011; Rodriguez-Manzano and others 2012), and in general bacteria are poor indicators of the presence of viruses and parasitic protozoa (Rimhanen-Finne and others 2004; Jurzik and others 2010; He and others 2012; Agullo-Barcelo and others 2013). As such, the existence of a linear relationship between the concentration of an indicator bacterium and, for example, a specific enteric virus is highly unlikely, particularly a pathogen with a distinctive seasonality and prevalence. The lack of a fixed correlation between pathogen and (a single) bacterial indicator and, hence, the invalidity of these ratios have been highlighted by Cutolo and others (2012) and Silverman and others (2013). For example, the inutility of using *E. coli* results to model virus health risks associated with the reuse of domestic greywater has been recently demonstrated in a study by O'Toole and others (2012), with a finding of no statistical correlation between the presence of the indicator and viruses.

Another point in the use of these ratios is that assumptions have been suggested to alter these ratios: the original data in Grimason and others (1993) and Oragui and others (1987) comprised fecal coliforms and several studies assumed to replace data of fecal coliforms with data of *E. coli* (Mara and Sleigh 2010b; Barker-Reid and others 2010) or total coliforms (Munoz and others 2010). Mara and Sleigh (2010b) assumed that noroviruses could be represented by enterovirus concentrations (Table 2), despite the distinct variability in seasonality of human NoV and enteroviruses. These pathogen/indicator ratios have also been used to estimate pathogen contamination of fresh produce. In studies by Forslund and others (2010, 2012) and Seidu and others (2008), the ratio was used to estimate rotavirus concentrations on potatoes, tomatoes, and lettuce that were all irrigated with (treated) wastewater. The validity of this practice is questionable, especially in the case of Seidu and others (2008), because the use of poorly treated poultry manure and cow dung as fertilizer is common practice in Ghana. These nonhuman waste materials would not be expected to harbor enteric viruses, and use of these ratios under these circumstances could lead to an overestimation of rotavirus concentration on produce. Taken together, extrapolation of relationships found in a specific wastewater system to other regions, other water sources, and other matrices (such as on fresh produce) should be approached cautiously. Consequently, critical evaluation of QMRA outcomes that result when using these ratios is necessary and can help identify faulty assumptions. This was observed by Barker-Reid and others (2010), who found overestimation of enteric virus risk associated with consumption of brassicaceous vegetables that were irrigated with greywater derived from kitchens. This higher risk was a consequence of the use of fecal coliform indicator ratios as proxy for enteric viruses, with elevated levels of the former likely associated with a nonhuman source of fecal contamination, perhaps from the washing of chicken carcasses.

A *fourth strategy* to address the lack of data on pathogen prevalence and concentration is the fecal loading approach. This resembles the previous approach as it, likewise, circumvents the constraints of analyzing large sample sizes of water for the presence of a specific pathogen. However, in contrast to the previous approach, it does not require extrapolation of experimentally determined ratios, but relies on the use of a reasoned calculation. This rationale requires, first (indirectly), the fecal loading of the potential irrigation water by, for example, use of a determined amount of *E. coli* per g (human) feces (Barker and others 2013) in greywater or by assuming that all *E. coli* in river water are originating from feces of herds harboring zoonotic pathogens such as verocytotoxin-producting *E. coli* (VTEC), as mentioned by Ottoson and others (2011). Next, a known pathogen-shedding concentration in feces (number of NoV particles/g feces in Barker and others 2013) or a known pathogen-to-*E. coli* ratio in feces (such as VTEC/*E. coli* in an infected herd and the proportion of infected herds) is needed. Thus, the NoV concentration in domestic greywater in Australia (Barker and others 2013) and the VTEC concentration in surface water contaminated by cattle herds in Sweden (Ottoson and others 2011) could be calculated. A downside of this approach is the need for many input data for the construction of the exposure model, which likewise increases the complexity and may introduce greater uncertainty in the final estimate of pathogen concentrations (Mok and others 2014). The latter was observed during sensitivity analysis for both studies (Ottoson and others 2011; Barker and others 2013), as the norovirus shedding rate and the VTEC/*E. coli* ratio in manure were responsible for the majority of the variability in probability of infection or illness.

A *final (fifth) option* to deal with lack of data is to simulate the initial contamination and/or prevalence of the pathogen for irrigation water/incoming product by the use of different potential scenarios (Danyluk and Schaffner 2011) or the use of one assumed scenario (van Ginneken and Oron 2000; Oron and others 2010; Table 2). In some QMRA studies, different (potential/existing) water/produce quality guidelines concerning maximum concentration of *E. coli* are selected in order to verify the validity of these guidelines to reduce the risk of defined pathogen exposure. Hence, the conversion to pathogen concentration is done according to the third approach using ratios or by the fourth approach using fecal loading. In some studies, scenarios using different pathogen concentrations in irrigation water were simulated in order to obtain maximum tolerable estimates of pathogens that would comply with certain acceptable maximum risk levels (Mara and others 2007; Navarro and Jiménez 2011).

Table 3–Data needs identified to model the transmission routes describing how pathogens are transferred from environment (water) to the crop and identified approaches (based on genuine data, surrogate [S] data or assumptions [A]) to deal with these data needs.

Knowledge on the transfer of pathogens during irrigation on farm level and effect of different irrigation strategies on contamination level of fresh produce.		
(i)	Use of **surrogate data** (based on limited experiments) to estimate the amount of water clinging to the crop after spray irrigation (V_{prod}), data can be based on:	
	– submersion experiments:	
	• Shuval and others (1997): Lettuce: 10.8 mL/100 g; cucumber: 0.36 mL/100 g	Mota and others (2009); Petterson and others (2011); Shuval and others (1997)
	Variants on this base data have been applied:	
	➔ Lettuce: Normal, μ=0.108, σ=0.019 (truncated at 0) (mL/g)	Ayuso-Gabella and others (2011) ; Barker and others (2013); Hamilton and others (2006a, b); Ottoson and others (2011)
	Uniform (8.9, 12.7) (mL/100 g)	Lim and Jiang (2013)
	Range: 10-15 mL/100 g	Diallo and others (2008); Mara and others (2007); Mara and Sleigh (2010b)
	Range: 10.8-15 mL/100 g	Drechsel and Seidu (2011)
	V_{prod} = 5 mL on 40 g of lettuce	NRMMC-EPHC-AHMC (2006)
	➔ Cucumber: Normal, μ=0.0036, σ=0.0012 (truncated at 0) (mL/g)	Hamilton and others (2006a, b)
	Uniform (0.24, 0.48) (mL/100 g)	Lim and Jiang (2013)
	• Bartz (1988) : tomato: 0.04-1.66 mL/100 g -> Uniform (0.04-1.63) mL/100 g	Lim and Jiang (2013)
	– Spray-irrigation experiments => Hamilton and others (2006a):	
	• broccoli (n=100): Log Logistic, α=4.246, β=1.583x10^{-2}, λ=1.085x10^{-3} (μ=0.0185)	Hamilton and others (2006a, b)
	• Savoy King/Grand Slam cabbage (2 x n=20): Empirical CDF (μ=0.0352) (mL/g)	Hamilton and others (2006a, b)
	• Winter head cabbage (n=20): Empirical CDF (μ=0.0889) (mL/g)	Hamilton and others (2006a, b)
	When no specific surrogate data was available, some studies used data as stated above as **surrogates** for other types of vegetables and fruits. E.g.	
	– The use of remaining water volume on cucumber, for other 'smooth produce' such as tomatoes and bell peppers	Mota and others (2009)
	– The use of remaining water volume on lettuce as surrogate for other leafy vegetables, and remaining volume of water on cucumber as surrogate for other vegetables and fruit	Munoz and others (2010)
	– The use of V_{prod} for Winter head cabbage as surrogate for all brassicaceous vegetables	Barker-Reid and others (2010)
	This approach has as inherent **assumption** that any microorganisms contained in the residual wastewater/irrigation water remaining on the irrigated vegetables would cling to the vegetables even after the wastewater itself evaporated.	
(ii)	If no relevant surrogate data is available, **assumptions** have been used for the amount of water clinging to crops after irrigation. For example:	
	• Assuming an average daily dose of 10 mL when eating vegetables	Asano and others (1992); Tanaka and others (1998)
	• Assuming that the volume of irrigation water remaining on onion is 1-5 mL per 100 g onions	Mara and others (2007)
	• Assuming 3-5 mL of irrigation water remaining on 100 g carrots	Mara and others (2010a)
	• In van Ginneken and Oron (2000) assumptions have been made on the amount of water remaining on fruits and vegetables when three different irrigation techniques were used:	
	– Spray irrigation: 16 mL/100 g	Van Ginneken and Oron (2000)
	– Onsurface drip irrigation: ~triangular distribution (min.: 0.016 mL/100 g; max.: 1.6 mL/100 g; mean: 0.16 mL/100 g of plant matter)	Oron and others (2010); Van Ginneken and Oron (2000)
	– Subsurface drip irrigation: ~triangular distribution (min.: 0.0016 mL/100 g; max.: 0.16 mL/100 g; mean: 0.016 mL/100 g)	Oron and others (2010) ; Van Ginneken and Oron (2000)
(iii)	The effect of the relevant type of irrigation water under study on the contamination level of fresh produce was examined during a *field experiment*. The level of microbial contamination of the crops (mostly fecal indicators) under study were determined upon harvest and used as input data of the QMRA model.	Finley and others (2009); Forslund and others (2010, 2012); Seidu and others (2008)
(iv)	The performance of field trials using surrogate microorganisms to **model** the transfer of specific pathogens in irrigation/pesticide water to the specific crop. Surrogates that are used:	
	– Coliphage PRD and *E. coli* ATCC 25922 as surrogate for the transfer of HAV and *Salmonella*, respectively	Stine and others (2005, 2011)
	– *E. coli* as surrogate for the transfer of *Giardia*, *Cryptosporidium*, rotavirus and *Campylobacter*.	Bastos and others (2008); Pavione and others (2013)
Additional studies on the effect of different irrigation water strategies on the pathogen load of different crops.		
(additional validation) studies on occurrence and effect of cross-contamination during farm-to-fork chain (washing, cutting, packaging). Including data on distribution of cross-contaminated microorganisms in processed produce.		
(i)	The use of data based on experiments to simulate the transfer of the pathogen to the crop. Some studies use data obtained using surrogate microorganisms others data based on cross-contamination studies that used the relevant pathogen under study. For example:	Ding and others (2013); Rodriguez and others(2011)
	– The use of nonpathogenic surrogates (PRD1 phage and *E. coli*) instead of the pathogens under study (HAV and *Salmonella*) to determine the fraction of microorganisms present on surface of cantaloupe that are recovered from the flesh after cutting the cantaloupe.	Stine and others (2005, 2011)
	– Danyluk and Schaffner (2011) modeled the cross-contamination of leafy greens with *E. coli* O157:H7 due to washing at processing stage using data of Zhang and others (2009) on the relevant pathogen under study.	Danyluk and Schaffner (2011)
(ii)	When no relevant data are at hand, the effect of cross-contamination can be simulated using different cross-contamination scenarios (with e.g. different types of distribution of bacterial load and different cross-contamination levels).	Puerta-Gomez and others (2013)
(iii)	Assuming that no cross-contamination of fruits and vegetables after harvest is occurring and therefore contamination of the crops is solely due to farm level contaminations	Al-Juaidi and others (2010); Oron and others (2010)

Filling the data gap regarding pathogen transfer during irrigation and washing of fresh and fresh-cut produce. *Transfer from irrigation water to the crop*. In simulating contamination routes, data necessary to model the transfer of pathogens from the (production) environment to fresh produce are needed. Several factors can contribute to the likelihood and degree of pathogen contamination to fresh produce at this phase, including water used for irrigation or insecticide/fungicide treatment; soil and insufficiently composted manure or biosolids used as fertilizers; wild or neighboring domestic animals or livestock grazing on adjacent fields (and associated run-off water); harvest or washing equipment; and field workers (Ilic and others 2012; Olaimat and Holley 2012; Liu and others 2013). Of these, contaminated irrigation water has received the greatest attention. However, food handlers, particularly those for hand-picked products, have been identified as important contributors to the overall microbiological quality of fresh produce (Leon-Felix and others 2010). So has contamination with pathogens present in soil/biosolids (Seidu and others 2008). The latter is particularly relevant to developing countries in which poorly treated manure or biosolids/sludge are still used as fertilizer. These 2 risk factors (food handlers and biosolids) should ideally be included as a source of contamination in fresh produce production. But the present review focuses on water as a contamination route and, therefore, only the approaches taken and assumptions made for modeling transfer from irrigation and washing water to the food crop were analyzed (Table 3) and are further discussed.

Different strategies have been used to model or estimate the number of pathogens on (the surface of) the crop after irrigation. *A first strategy* is to use surrogate data to estimate the amount of water clinging to the crop after irrigation and assuming that any microorganisms contained in the residual water remaining on the edible product would cling to the vegetables, also after evaporation of the water. Estimation of degree of contamination can be estimated using this approach if the microbial load of the water is known and if an estimate is at hand of the amount of irrigation water retained by the produce (van Ginneken and Oron 2000; Oron and others 2010). Often, estimates of the amount of water retained on the produce are based on surrogate data originating from one single study, such as by Shuval and others (1997), in which the worst-case scenario was simulated by total immersion of pre-weighed cucumbers ($n = 26$) and lettuce heads ($n = 12$) in water. These results (lettuce: on average 10.8 mL/100 g; cucumber: on average 0.36 mL/100 g), which were originally presented as point-estimates, have been commonly used in studies that assessed the transfer of pathogens by spray-irrigation (Lim and Jiang 2013). Hamilton and others (2006a) imposed a normal distribution on these data (used also by Barker and others 2013; Ottoson and others 2011). In addition, Hamilton and others (2006a) determined the amount of water retained on some other vegetable products. In this study, the amount of water retained on broccoli ($n = 100$) and 3 cultivars of cabbage ($3n = 20$) was determined in field tests using overhead irrigation. The resulting distributions have served as input data for other QMRA studies (Hamilton and others 2006b; Barker-Reid and others 2010). These limited data for lettuce, cucumber, broccoli, and cabbage have been used as stand-in data for other vegetables (Mota and others 2009; Munoz and others 2010). Recently new data have been published concerning the volume of irrigation water captured after irrigation of lettuce (green oak lettuce) and Asian vegetables, such as Chinese chard, Chinese broccoli, and Chinese flowering cabbage (Mok and Hamilton 2014). When different crops are compared, the risks for lettuce tend to be higher because of this product's relatively higher water retention rate (Hamilton and others 2006a; Lim and Jiang 2013).

In the absence of data, a *second approach* is to use assumptions to estimate the amount of water adhering to crops after irrigation. This was done in 2 of the oldest QMRAs included in this review (assuming an average daily exposure of 10 mL water; Asano and others 1992; Tanaka and others 1998). Some studies have also used this approach to estimate the microbial load of root vegetables such as onions (Mara and others 2007) and carrots (Mara and Sleigh 2010a). In studies by Oron and others (2010) and van Ginneken and Oron (2000), assumptions were made on the amount of water retained on the crop (fruits and vegetables), differentiating between spray irrigation, drip irrigation, and subsurface drip irrigation (Table 3).

A third approach to fill the data gaps about pathogen transfer from water to produce has been to use data from field experiments in which the produce had been irrigated during growth with the relevant type of irrigation water under consideration (with naturally occurring microorganisms). The subsequent microbial load of the irrigated crops is then determined upon harvest and used as input data in the QMRA model. The first study taking this approach was that of Seidu and others (2008), who used data from previous studies by Amoah and others (2007a, 2007b) and Obuobie and others (2006). These studies assessed the concentration of *Ascaris* and fecal coliforms on lettuce irrigated with different water types (drain, stream, and piped water). For the QMRA of Finley and others (2009), field data on the contamination level of lettuce, carrots, and peppers irrigated with (treated) greywater or tap water at soil level was obtained for fecal coliforms and fecal streptococci. In Forslund and others (2010, 2012), field studies were performed to assess the contamination of potatoes and tomatoes with *E. coli* after the use of different-treated waters and irrigation methods. Note that in all of these field studies, except for the detection of *Ascaris*, fecal indicator bacteria were monitored, after which in all but one study, the one by Finley and others (2009), a pathogen/indicator ratio was used to estimate the amount of rotaviruses on the crop. As such, the fecal indicator bacteria data were used as proxy for the transfer and attachment of viruses.

The last and *fourth approach* is also based on field data. In this case. Field trials were performed not simply to use the data, but rather to simulate the transfer of the pathogens in (irrigation) water to the crop via the development of a formula to calculate transfer rate. In 2 studies done by Stine and others (2005, 2011), field trials were conducted to obtain the percentages of microorganisms transferred from water to the surface of fresh produce via irrigation and by application of water-diluted pesticide spray, respectively. In both studies, the coliphage PRD1 and *E. coli* ATCC 25922 were used as surrogates for the transfer of HAV and *Salmonella*, respectively. Two different irrigation methods (subsurface drip irrigation and furrow irrigation) were evaluated in the field studies and both trials were performed on 3 produce types: cantaloupe, iceberg lettuce, and bell peppers. Bastos and others (2008) performed field trials to obtain formulas for low-growing crops and high-growing crops that related the concentration of *E. coli*/100 mL irrigation water with the *E. coli* concentration per gram on the crop. In this case, watering was done using cans and *E. coli* was used as a surrogate for *Giardia, Cryptosporidium*, rotavirus, and *Campylobacter*. These data were later also used by Pavione and others (2013).

The latter 2 approaches (using data from field trials) have an advantage in that the effect of repeated irrigation with contaminated water and attachment, survival and growth of the surrogate organisms during production, are included in the estimates. In

contrast, the first 2 approaches only take into account the amount of water clinging to the crop after one irrigation event (or water submersion) after which survival is included during the subsequent withholding period (period between last irrigation and harvest). However in a recent study by Mok and others (2014), the possible accumulation of the pathogen on the crop during consecutive irrigations was included in the model that used the first approach for modeling viral transfer. A downside of the latter 2 approaches is that the data may not be very applicable to other situations, as different environmental and climatic conditions could influence the final microbial loads. In several field studies, the influence of crop type was investigated by selecting a root, leaf, and fruit crop each of varying heights (Stine and others 2005; Bastos and others 2008; Finley and others 2009; Stine and others 2011).

Transfer of microorganisms/cross-contamination during postharvest rinsing and washing. Contamination of produce because of cross-contamination during packing/processing (including washing) has also been modeled, albeit in a limited number of studies (Danyluk and Schaffner 2011). There is increasing interest in this phenomenon because of the rising market for prepackaged, washed salad vegetables. Validation studies to characterize transfer/cross-contamination rates during industrial processing or salad preparation has been identified as a data gap (Danyluk and Schaffner 2011; Rodriguez and others 2011; Puerta-Gomez and others 2013). Recent studies evaluated cross-contamination from nylon brushes and peelers that were contaminated with viruses to uncontaminated carrots and celery (Wang and others 2013), and on cross-contamination of lettuce with bacteria (*E. coli*) and viruses (MS2 and MNV-1) during simulation of industrial washing procedures of fresh-cut lettuce washing (Holvoet and others 2014).

Filling the data gaps on reduction/growth/survival of microorganisms along the fresh produce production chain. *Growth and survival.* As a result of the frequent unavailability of relevant pathogen survival data on fresh produce, surrogate data and assumptions have mostly been used in selected QMRAs. However, the preferred *(first) approach* is the use of actual growth curves or studies on persistence obtained for the relevant pathogen on the specific produce item under consideration, such as by Stine and others (2005, 2011). Yet in some cases the use of surrogate data or surrogates *(second approach)* is more practical or even a necessity. The latter is the case, for example, for the noncultivable human norovirus (Knight and others 2013). Petterson and others (2001a), for example, used *Bacteroides fragilis* bacteriophage B40-8 as a surrogate for human enteroviruses to estimate their survival on lettuce. *B. fragilis* phage B40-8 was chosen because it is considered as a conservative model for human enteric viruses and may be expected to be inactivated at a slower rate than the human viruses (Petterson and others 2001b). The resulting first-order decay constant ($k = 1.07$ per day, $\sigma = 0.07$) has been used in several other studies (Al-Juaidi and others 2010), including some that used this model to represent survival of enteric viruses on other types of produce (Hamilton and others 2006a, 2006b). Decay or the loss of viability/infectivity of pathogenic microorganisms is traditionally modeled, assuming a simple first-order kinetic model where the decay constant is affected by various environmental factors (temperature, solar radiation, relative humidity, and presence of inhibiting/inactivating substances). However, simple first-order (single-phase) die-off is probably not accurate as most soil and subsurface environments are highly heterogeneous and because of the potential for long-term survival of persistent subpopulations and/or re-growth in the environment (Bradford and others 2013). As such, biphasic survival kinetics have been observed in both water (Easton and others 2005; Ahmed and others 2014) and fresh produce production environment (Petterson and others 2001b; Seidu and others 2013). In biphasic decay kinetics an initial rapid decay is noted, often followed by an attenuated, slower decay. The inclusion of the possibility of a biphasic decay in QMRA is important as the survival of pathogens, and hence the predicted infection risk, can be significantly underestimated if the presence of a persistent subpopulation of the microorganisms is not considered (Petterson and Ashbolt 2001; Seidu and others 2013).

A *third approach* is the use of estimates or assumptions on log reductions of the pathogen on the crop/plant that takes place during plant growth in the field or postharvest. Some studies have used estimates for log reductions, whether or not combined with removal by washing/disinfection (during consumer preparation; such as used by Shuval and others 1997; Pavione and others 2013). An assumption that has been frequently used is that for the survival of enteric viruses, a first-order decay as a function of time ($\mu_1 = \mu_0 \times e^{(-kt)}$) is appropriate, with a generic decay constant k of 0.69 per day (Asano and others 1992; Munoz and others 2010). This constant is primarily used to model decay during the withholding period in the field; however, the exact provenance of this virus decay rate coefficient is unknown (O'Toole 2011). Hence, there is no indication whether this decay rate is substantiated by results from independent studies and therefore the use of this constant was labeled as an assumption in Table 4. This same decay constant ($k = 0.69$ per day) was also used in an early risk assessment dealing with the decay of viruses in an Illinois river (Haas 1983). But after sensitivity analysis, Haas (1983) concluded that variation in this decay constant resulted in the greatest variation in the resulting risk estimate and, hence, particular attention should be paid to obtain data on viral decay in order to develop more precise estimates of risk. A similar conclusion about the importance of the selected decay model/constant was drawn by Hamilton and others (2006a), Petterson and others (2001a), and Seidu and others (2013).

As a last *(fourth) approach*, some studies assumed pre- and/or postharvest decay to be negligible (Hamilton and others 2006a; Mota and others 2009). Many of these QMRA articles relate to viruses, and viruses are inert and relatively stable under common (assumed) storage conditions of fresh produce. However, enteric bacterial pathogens such as *Salmonella* and pathogenic *E. coli* may have the ability to multiply (or die) on fresh-cut produce, depending upon storage conditions. As there is an increasing trend towards buying prepacked leafy greens with shelf-lives up to 2 wk or longer, parameters such as microbial survival and growth throughout the farm-to-fork continuum should be taken into account (Ottoson and others 2011). Some studies did include survival/growth during storage at retail, in food service operations or at home (Carrasco and others 2010; Puerta-Gomez and others 2013).

Removal and inactivation. Consideration of postharvest inactivation and removal strategies such as washing (with or without the use of sanitizers in the water), irradiation, or peeling were often absent in QMRA studies, predominantly because half of the studies just did not include further processing or consumer preparation in the model. A few studies ($n = 9$) considered washing at the consumer phase (Ottoson and others 2011; Barker and others 2013) and in some cases ($n = 6$), at the processing level (Carrasco and others 2010; Rodriguez and others 2011).

The potential of human pathogens to become internalized within growing vegetables is of concern because when residing in internal locations, the organisms become more difficult to

Table 4–Data needs related to growth/survival/removal/inactivation of microorganisms along the fresh produce chain and identified approaches (using genuine data, surrogate (S) data, or assumptions (A)) to deal with these data needs.

Specific survival/growth data for very specific situation (survival in pesticide spray, crop-specific survival) or for the pathogen under study is often missing		
(i)	The use of growth and/or survival data obtained for the relevant pathogen on the specific crop under study, ideally when simulating relevant environmental conditions.	Ding and others (2013); Puerta-Gomez and others (2013); Stine and others (2005, 2011)
(ii)	The use of data based on experiments in which a **surrogate** microorganism was used instead of the specific pathogen under study or the use of data based on experiments performed on a different type of produce then the produce under study, to study survival/growth of a specific pathogen on a specific crop. For example:	
	–use of *Bacterioides fragilis* bacteriophage B40-8 as a surrogate for enteroviruses or other enteric viruses	Al-Juaidi and others (2010); Barker and others (2013); Petterson and others (2001a)
	–use of survival data obtained for lettuce for another produce: for example cucumber, broccoli, and cabbage	Hamilton and others (2006a, b)
	Models are often constructed assuming a simple first-order kinetic model.	
(iii)	The use of **estimates** or **assumptions** on log reductions of the pathogen on the crop at pre-harvest and/or post-harvest stage.	
	–Estimates for log reductions comprising survival on field but also removal/inactivation steps further in the chain (e.g. washing by consumer)For example:	
	• Assumption of total virus inactivation/removal of 3 or 2-3 logs as rough, conservative estimation for inactivation on field and/or as estimate for removal by e.g. washing and survival post-harvest	Mara and others (2007); Pavione and others (2013); Seidu and others (2008); Shuval and others (1997)
	–Assumptions, for example:	
	• Assumption of a first order decay with as decay constant k = 0.69 d^{-1} for enteric viruses	Asano and others (1992); Hamilton and others (2006a); Munoz and others (2010); Oron and others (2010); Tanaka and others (1998); van Ginneken and Oron (2000)
(iv)	**Assuming** pre- and/or post-harvest decay or effect of storage to be negligible.	Barker-Reid and others (2010); Bastos and others (2008); Mara and Sleigh (2010a); Mota and others (2009); Ottoson and others (2011); Stine and others (2011)
Specific experimental data on the removal/inactivation of the pathogen		
(i)	The use of removal/inactivation data obtained for the relevant pathogen on the specific crop under study.	Doménech and others (2013) ; Puerta-Gomez and others (2013)
(ii)	The use of data based on experiments in which a **surrogate** microorganism was used instead of the pathogen under study or which was performed on a different type of produce to study the effect of removal and/or inactivation strategies (e.g. washing) of a specific pathogen on a specific crop.	Barker and others (2013); Carrasco and others (2010)
(iii)	The use of **estimates** or **assumptions** on log reductions of the pathogen on the crop (the same as for survival/growth).	
	For example:	
	– Assumption of total virus inactivation/removal of 3 or 2-3 logs as rough, conservative estimation for inactivation on field and/or as estimate for removal by washing and survival post-harvest	Pavione and others (2013) ; Shuval and others (1997)
	– Assuming that the combined effect of washing (1 $\log_{10}$ reduction (WHO (2006)) and disinfection (2 $\log_{10}$ reduction (WHO (2006))) during salad preparation would lead to a 3 $\log_{10}$ reduction of rotavirus.	Seidu and others (2008)
(iv)	**Assuming** that post-harvest removal/inactivation is negligible (worst-case scenario).	Barker-Reid and others (2010); Bastos and others (2008); Forslund and others (2012); Mota and others (2009)

remove by physical washing, or to inactivate using surface sanitizers. Several studies have demonstrated the internalisation of pathogenic *E. coli* or *Salmonella* in leafy greens harvested following cultivation on contaminated manure-amended soil and irrigation water (Solomon and others 2002; Bernstein and others 2007; Ongeng and others 2011; Pachepsky and others 2011). Zheng and others (2013) demonstrated that both infested soil and contaminated blossoms can lead to low internal levels of tomato fruit contamination with *Salmonella*. Human norovirus RNA was detected in lettuce leaves after exposure of the roots to the virus particles (DiCaprio and others 2012). Oron and others (2010) recovered poliovirus from leaves (but not in the fruit) after growth of tomato plants in soil irrigated with poliovirus-contaminated water. Golberg and others (2011) demonstrated that internalization of *Salmonella* Typhimurium through leaf epidermis is variable in leafy greens. Various factors have been shown to affect the ability of human pathogens to internalize, including growth substrate (soil vs. hydroponic solution), plant developmental stage, pathogen genus and/or strain, inoculum level, and plant species and cultivar (Hirneisen and others 2012). In general, internalization in leafy greens has been observed after inoculation of high levels of *Salmonella* and other human pathogens, making difficult an assessment of its importance in real conditions (Warriner and Namvar 2010). The possible internalization of pathogens inside the plant tissue can also be considered as a data gap and could be relevant to consider in assessing the effect

of washing or other decontamination strategies (Sales-Ortells and others 2015).

Again, different approaches can be taken for modeling pathogen removal/inactivation during washing or decontamination (Table 4). Similar to approaches described above, some QMRA studies use rough estimates of the reduction of pathogens by washing, often in combination with survival (Shuval and others 1997; Pavione and others 2013). Other studies have used crop-specific experimental data on the effect of washing on the produce and pathogen(s) under consideration (Doménech and others 2013; Puerta-Gomez and others 2013). The use of removal/inactivation data derived from experimental studies on other types of crops as proxy for a different product has been applied, such as a shift from Brussels sprouts to lettuce (Carrasco and others 2010). For other reasons, the use of surrogate data is sometimes a necessity, as is the case for noncultivable pathogens like noroviruses (Barker and others 2013).

Next to washing and/or disinfection, irradiation was also investigated as an inactivation strategy for *E. coli* O157:H7 and *Salmonella* spp. on fresh-cut bagged lettuce (Rodriguez and others 2011) and ready-to-eat baby spinach (Puerta-Gomez and others 2013), respectively. A crude estimation of pathogen reduction achieved by peeling has been considered in the case of carrots (Mara and Sleigh 2010a).

In general, pathogen growth, survival, removal, and inactivation data and predictive models have been identified as a data gap for QMRA, both pre- and postharvest (Table 4). In the preharvest phase, climatic conditions (such as temperature, solar radiation, or relative humidity) can influence the survival of pathogens in the field (Petterson and others 2001b). Survival of microorganisms has also been suggested to be crop-specific (Verhaelen and others 2012; Macarisin and others 2013) and could be affected by the competing microbiota present, which are impacted by many factors (Ottoson and others 2011) and internalization. Moreover, postharvest decay or growth along the farm-to-fork continuum is not always included in QMRA studies and can be relevant, in particular for bacterial pathogens, if longer shelf-lives are applied. Knowledge on survival and growth of pathogens on specific fresh produce commodities is accumulating and data on the use and performance of sanitizers to avoid cross-contamination during washing and decontamination of fresh(-cut) produce are becoming increasingly available. Some examples include Mansur and others (2014) who produced a growth model for *E. coli* O157:H7 on treated kale; Carratala and others (2013) who described survival of hAdV in water under different environmental conditions; Zeng and others (2014) who described growth of *E. coli* O157:H7 and *L. monocytogenes* in packaged fresh-cut Romaine mix at fluctuating temperatures anticipated during commercial transport, retail storage, and display; and Bozkurt and others (2014) who modeled thermal inactivation of human norovirus surrogates in spinach. Again, use of surrogates requires caution. Some surrogates commonly used for norovirus, such as feline calicivirus strain F-9, have been shown to be less tolerant to chlorine treatment and thermal processing (Topping and others 2009; Nowak and others 2011). In the discussion of growth, survival, removal, and inactivation the possible effect of internalization of pathogens inside the plant tissue can also be considered as a data gap and could be relevant for consideration (Sales-Ortells and others 2015). Internalization of human pathogens can occur through root uptake, through cellular structures (stomata), or wounds. This physical entrapment below the surface could function as protective shelters making postharvest treatments such as chlorine sprays and washes ineffective (Hirneisen and others 2012; Hirneisen and Kniel 2013).

Filling the data gap on consumer behavior. Consumer behavior, both practices and consumption patterns, influences exposure and hence risk (CAC 1999). Relevant fresh produce consumption data, including frequency and portion size for key populations, are essential for exposure assessment (Donne and others 2011; Hoelzer and others 2012). Such data have been used in QMRA studies (such as by Ferrer and others 2012; Table 5). However, relevant national consumption data are not always available for every country and, hence, data derived from other countries and/or populations are frequently used as proxy (Navarro and Jimenez 2011; Barker and others 2013; Table 5). Frequently used consumption data are those derived from the U.S. National Health and Nutrition Examination Survey (NHANES) and as further elaborated by the U.S. Environmental Protection Agency (U.S. EPA, 2011) examplified by the QMRAs of Navarro and others (2009) and Oron and others (2010), whereas a European database is not yet available and national consumption surveys are still applied (Jacxsens and others 2015). Consumption data derived from official institutes such as U.S. NHANES, U.S. EPA, or EFSA are often expressed as daily consumption (g/d), mostly to be used for nutritional purposes or risk assessments associated with chronic chemical exposure studies (Vinci and others 2012; De Boevre and others 2013). Of course, in QMRA in which acute exposure is the problem, average consumption over time is less relevant than the portion and frequency of consumption of a product (CAC 1999). It should also be noted that it is difficult to compare consumption data from different countries because of different data collection methods and resources that can go into such data collection (Donne and others 2011; EFSA 2013). Consequently, dietary surveys can differ with respect to a number of parameters affecting the level of detail and the accuracy of the collected data, such as: (i) the dietary assessment method, for example, 24-h recalls, food frequency questionnaires (FFQ), or via diaries; (ii) the number of days over which information is collected; (iii) sampling design; and (iv) method for quantification of portion sizes. In an effort to provide more standardization, European countries are engaged in an effort to harmonize collection of consumption data (EU project EU Menu http://www.efsa.europa.eu/en/datexfoodcdb/datexeumenu.htm). In the United States, a comprehensive study to obtain harmonized data on fresh produce consumption was recently completed (Hoelzer and others 2012). Specific populations, such as children and the elderly, can be particularly vulnerable to certain microbes and this should also be considered when using consumption data (Kroes and others 2002; EFSA 2009). Another approach for dealing with consumption data needs is to use assumptions about portion size (Petterson and others 2001a; Ottoson and others 2011) and/or consumption frequency (Shuval and others 1997).

For instance, in studies where actual risk estimates are not required, such as methodological studies (Petterson and Ashbolt 2001; Petterson and others 2001a) and studies whose main objective is to analyze the effect of different scenarios (different risk mitigation strategies) (Carrasco and others 2010; Ottoson and others 2011), the use of surrogate data or assumptions on consumption are acceptable. However, when an actual risk estimate is the objective, or when exposure to different crops is compared, relevant consumption data are a prerequisite. Indeed, it has been shown in sensitivity analyses that the amount of produce consumed, or serving size, can have an important effect on the uncertainty

Table 5–Data needs related to consumer behavior and identified approaches (using genuine data, surrogate (S) data or assumptions (A)) to deal with these data needs.

Specific consumption data of the situation (country/region) under study		
(i)	The use of relevant consumption data for the region/country and situation under study.	Bastos and others (2008); Ferrer and others (2012) ; Hamilton and others (2006b)
(ii)	The use of consumption data of another country as **surrogate** for the consumption patterns in the country relevant for the study (consumption size and frequency). For example:	
	– The use of consumption data derived from the US DA or US EPA for another country/situation.	Barker and others (2013); Mara and Sleigh (2010a); Navarro and others (2009) ; Oron and others (2010)
(iii)	The use of **assumptions** on consumption portions and/or frequencies. For example:	Finley and others (2009) ; Mota and others (2009)
	– 100 g of lettuce per person on alternate days or 150 days a year.	Mara and others (2007) ; Shuval and others (1997)
	– Each consumption event (of lettuce) comprises 100 g.	Ottoson and others (2011); Petterson and others (2001); Petterson and Ashbolt (2001)
Information on consumer practices such as the prevalence, frequency, or intensity of vegetable washing and cooking habits in the population under study		
(i)	The use of specific knowledge on household practices concerning the preparation of fresh produce in the community. For example:	
	– Knowledge of washing practices in a community	Barker and others (2013) ; Doménech and others (2013)
	– The use of specific consumption data of *raw* produce	Carrasco and others (2010); Ferrer and others (2012)
(ii)	The use of **assumptions** or scenario analyses. For example	
	– Assuming that the vegetables under study are all consumed raw (e.g. lettuce, cucumber, but also broccoli or cabbage).	Al-Juaidi and others (2010); Hamilton and others (2006a); Mota and others (2009); Navarro and others (2009)
	– Washing of produce by consumers was often included in scenario analysis (present or not), so true prevalence and efficiency was not accounted for.	Ottoson and others (2011)

surrounding a risk estimate (Hamilton and others 2006a; Carrasco and others 2010; Lim and Jiang 2013).

Consumption habits can be highly culturally dependent: a serving size of 85 g of cut leafy greens was used in a QMRA (Danyluk and Schaffner 2011) as a representative portion size for the United States, whereas a consumption portion of 10–12 g was used to model risks associated with raw salads that are mainly sold as "street-food" in Ghana (Seidu and others 2008). Mara and Sleigh (2010b) compared the effects of 2 different consumption patterns (100 g every 2 d and 10–12 g on each of 4 d a week) on the norovirus log reduction needed to comply with a tolerable level of risk associated with consumption of waste-water-irrigated lettuce. A one log difference was observed in pathogen reduction required along the farm-to-fork chain, depending upon the consumption pattern chosen for the risk modeling. Clearly, the application of one country's consumption data to another may not always be relevant. Another standard assumption made in QMRA is that all of the commodity consumed is produced under the conditions being modeled (for example, use of recycled water or wastewater at the farm level; occurrence of washing at the processing stage; NRMMC-EPHC-AHMC 2006; Pavione and others 2013). It is necessary to investigate the possible impact of defined risk mitigation strategies when compliance is 100%, but it does produce a worst-case scenario risk estimate.

Another limitation of most consumption data is the absence of information on the state of a food item at consumption (raw, washed, peeled, cooked, stir-fried, steamed; Agudo and others 2002; Soerjomataram and others 2010; EFSA 2013). The state of the product can be highly relevant to the actual risk estimation as several consumer practices can have major influences on the microbial characteristics of the product at the time of consumption. Such preferences can be culturally dependent, requiring region-specific data on household practices, such frequency or rigor of vegetable preparation/washing (Barker-Reid and others 2010; Pavione and others 2013) and the proportion of fruits and vegetables eaten raw. Such data are lacking in most QMRA studies. In the absence of more specific data on consumer behavior, the most frequently used assumption for QMRA is that the produce item(s) under study are all eaten raw (lettuce, cucumber, but also broccoli and cabbage; Hamilton and others 2006a). Studies can circumvent this problem by using consumption data specific to raw produce (Carrasco and others 2010), and for some vegetables, such as lettuce, it is reasonable to assume that most of the product will be consumed raw. For other vegetables such as broccoli, cabbage, spinach, and carrots, the assumption of raw consumption can be used as a worst-case scenario. Only 2 studies have tried to model the fraction of product eaten uncooked, unpeeled, and unwashed, in these cases by using a triangular distribution based on assumptions about the prevalence of such practices (van Ginneken and Oron 2000; Oron and others 2010). Several studies do include a washing step before consumption (Navarro and others 2009), but QMRAs that use specific data on the frequency or intensity of vegetable washing (such as in Barker and others (2013) and Domenech and others (2013) are scarce as vegetable washing is not usually characterized by degrees but rather by yes (washed) or no (not washed; Ottoson and others 2011). However, in the study of Doménech and others (2013) they used specific reduction data on the lettuce of the pathogen under study using varying dipping/rinsing times and different concentrations of sodium hypochlorite for disinfection, according to practices identified during a consumer behavior survey.

Selection of dose–response model in QMRA studies on water use in fresh produce. To calculate the risk of infection or illness, the selection of a dose–response model for use in QMRA is

Table 6–Data needs identified to model the dose–response relation and identified approaches (using genuine data, surrogate [S] data or assumptions [A]) to deal with these data needs.

Dose–response data on the pathogen under study	
(i) The use of dose–response models for the specific pathogen under study, that are based on:	
– information obtained during human feeding studies	Barker and others (2013); NRMMC-EPHC-AHMC (2006)
– data derived from epidemiological studies	
– animal experiments, if possible validated with outbreaks	Seidu and others (2013) for *E. coli* O157:H7
(ii) The use of surrogate dose–response models. For example:	
– To model the dose–response of 'enteric viruses', the group was treated as a single pathogen with a known dose–response model. Generally the dose–response model of rotaviruses is used, as rotaviruses have a low infectious dose and as such represent a worst-case situation.	Al-Juaidi and others (2010); Barker-Reid and others (2010); Hamilton and others (2006a); Munoz and others (2010)); Petterson and Ashbolt (2001); Petterson and others (2001); Tanaka and others (1998); van Ginneken and Oron (2000)
– The use of the dose–response model of *Shigella dysenteria* as a surrogate for the dose–response model of *E. coli* O157:H7.	Danyluk and Schaffner (2011); Ottoson and others (2011)
– The use of the dose–response model of *Entamoeba coli* as a surrogate for the dose–response model of *Entamoeba histolytica*.	Ferrer and others (2012)
(iii) When no dose–response studies or estimates/surrogates for the dose–response model are available, the use of a worst-case situation can be appropriate. This worst-case situation can be modeled by, for example, the use of the exact single-hit model with probability of infection = 1 (r = 1), which represents the maximum risk curve.	Seidu and others (2008)

essential. Different dose–response models were used in the literature selected for review, including the exponential model (as in Mota and others 2009), the β-Poisson model (as in Lim and Jiang 2013), the approximated β-Poisson model (as in van Ginneken and Oron 2000; Petterson and others 2001a, b), the β-binomial model (as in Hamilton and others 2006a; Barker-Reid and others 2010), and the Weibull-Gamma model (Carrasco and others 2010). Each model, of course, has its own inherent assumptions, for example on the distribution of the received dose and/or on the distribution of infection (Vose 2008). For some pathogens, different dose–response models were selected in different studies: for *Salmonella*, a β-Poisson dose–response model was selected by some studies (Stine and others 2005; Drechsel and Seidu 2011; Lim and Jiang 2013) and an exponential model was chosen by another (Puerta-Gomez and others 2013). The preferred approach is the use of dose–response models that are based on information obtained during challenge (feeding) studies in human volunteers. However, for certain pathogens there are no feeding studies (usually because of ethical reasons), and in this case data can also be derived from epidemiological studies (or from animal experiments; Kothary and Babu 2001). Nevertheless, a relevant dose–response model was simply not available for all pathogens under consideration (Table 6). Moreover, because of the limited available data to construct a dose–response model, surely in the lower dose area, often huge uncertainties in the dose–response relationship are present, which may impact the final outcome of a QMRA study. Moon and others (2013) proposed mathematical solutions to include these uncertainties into the dose–response modeling.

An alternative approach is (again) the use of surrogate dose–response models. For example, in the case of QMRA studies for "enteric viruses," this group of diverse viruses was treated as a single pathogen with a given dose–response model, for example, rotavirus (Tanaka and others 1998; Barker-Reid and others 2010), used in QMRA. As rotavirus was considered at the time to be the most infectious water and foodborne virus for which dose–response information was available, its use in this modeling was justified as providing a plausible upper-limit to the risk estimates (Haas and others 1993). However, with the recent availability of dose–response models for norovirus based on human challenge studies, norovirus may be a better "reference" viral pathogen in the future (Teunis and others 2008; Mara and others 2010). Another example is the use of the dose–response model of *Shigella dysenteriae* and *Entamoeba coli* as surrogates, respectively, for *E. coli* O157:H7 (Danyluk and Schaffner 2011; Ottoson and others 2011) and *E. histolytica* (Ferrer and others 2012).

If no dose–response studies or estimates/surrogates for the dose–response relationship are available, a worst-case can be considered for modeling purposes. This was the case in the QMRA study done on *Ascaris* by Seidu and others (2008). In this study, a worst-case situation was assumed by using the exact single-hit model with probability of infection = 1 (r = 1; Teunis and Havelaar 2000). A different approach was undertaken by Navarro and others (2009), who developed a dose–response model for *A. lumbricoides* concerning likelihood of infection in children (under 15 y old) from crops eaten raw (irrigated with wastewater). In this case, prevalence data obtained from stools of a large sample of children

in the Mezquital Valley in Mexico were used in conjunction with assumptions (for example, on consumption) and surrogate data (for example, to estimate amount of water remaining on produce and, hence, crop concentration). This dose–response model was also used in several other QMRAs (Mara and Sleigh 2010a; Navarro and Jimenez 2011; Seidu and others 2013). An overview of these approaches is provided in Table 6.

To facilitate collection of data for dose–response modeling, most of the studies selected assumed that all strains of a certain pathogen are pathogenic/infectious to humans (Carrasco and others 2010; Ottoson and others 2011; Lim and Jiang 2013). This can be considered as a worst-case scenario as infectivity/pathogenicity for some microorganisms (such as for *Salmonella* and *E. coli*) is indeed strain-specific (Ceuppens and others 2013; Leimbach and others 2013) and characteristic of the host (age, immune status, physical condition) (Kothary and Babu 2001). To take this into account, some studies have assumed 25% as a preliminary estimate for a reasonable range of the parameter values in infection probability for use in Monte Carlo simulation (Mara and others 2007). For *L. monocytogenes*, a difference in susceptibility has been dealt with by using different parameter values for high-risk and low-risk populations (Carrasco and others 2010; Ding and others 2013). The risk of cryptosporidiosis in immunocompromised people (such as HIV-infected individuals; Howard and others 2006) associated with park irrigation with reclaimed water was calculated assuming a minimum infective dose of 1 of 10 the dose for healthy individuals (Ayuso-Gabella and others 2011). Other strategies for inclusion of the immunocompromised subpopulation in dose–response modeling have been used in studies outside the scope of this review (Howard and others 2006; An and others 2011). In the case of viruses, dose–response models suffer also from difficulties in assigning the ratio between infectious and defective particles (Bouwknegt and other 2015).

A systematic assumption for dose–response models used in QMRA is that different exposure events are independent, hence there is no protective immunity in the target population, (as mentioned by Ayuso-Gabella and others 2011). This may be particularly important when using these models for estimating disease risk in developing countries, as the dose–response models for almost all pathogens are based on data collected from developed countries (Ferrer and others 2012). Populations of developing countries tend to experience higher exposure to many pathogens and, consequently, high levels of immunity to certain pathogens may develop early in life (such as for HAV and enteroviruses; Hamilton and others 2006b). Navarro and others (2009) discussed the applicability of using their β-Poisson dose–response model for *A. lumbricoides* that was based on underlying data obtained from children in a population in which Ascariasis was endemic. They brought up that this model might not be directly applicable to a healthy population considering underlying cases of immunity in the test population. However, attempts have been made in a study outside the scope of this review to include the effect of population immune status in dose–response modeling (Teunis and others 2002a; An and others 2011).

Another observation about dose–response models is that for most of the pathogens, dose–response data are available for a single isolate only. Volunteer studies with different *Cryptosporidium parvum* isolates indicate that different isolates may produce different dose–response data and functions (Teunis and others 2002b).

A final consideration on the infectious disease calculations and inclusions of dose–response is the fact that secondary infection is usually ignored in food-related QMRAs, and a single individual can be infected with a particular pathogen only once per year. Food-related QMRA could be complemented with other models, such as the susceptible-infected-susceptible (SIS) model of disease transmission in order to calculate a more realistic impact on human health by including not only the consumer of the food or water, but also the surrounding "network" of the patients. Such network models are well established for other infectious diseases such as AIDS or malaria (Samatha and Chattopadhyay 2014).

Expression of risk estimate and benchmarking to an acceptable level of protection

Among the 41 QMRA studies analyzed, some benchmarked their outcome to a defined objective or acceptable level of protection, whereas others provided the comparison of the annual probability of infection or illness using various scenarios or risk mitigation strategies as an outcome. The former appears to be more common in QMRA studies dealing with water treatment or water quality relative to QMRA studies dealing with food (Table 7). In QMRA studies elaborated from the water perspective, one often refers to the benchmark level of acceptable risk once defined by the U.S. EPA in its water standards (U.S. EPA 1989). The U.S. EPA considered at the time one infection per 10000 individuals in a given year ($\leq 10^{-4}$ per person per year or abbreviated as $\leq 10^{-4}$ pppy) as a reasonable level of safety of drinking water. This number was derived in 1987 by determining the waterborne disease burden already tolerated in the United States. The total number of reported cases of waterborne illness per year (then estimated to be 25,000) divided by the U.S. population (250000000 at the time; Lechevallier and Buckley 2007). Another often cited acceptable risk level among the selected QMRA studies from a water microbiology perspective is limiting the maximum additional burden of water- and wastewater-related disease (provoked by use of reclaimed water) to 10^{-6} Disability Adjusted Life-Years (DALY) loss pppy (WHO 2004). The DALY metric has been introduced to enable comparison between the public health impact of various agents (microbial or chemical) and intervention options (Havelaar and Melse 2003). Both acceptable risk levels originate from WHO's water guidelines, and are integrated in QMRA studies on the risk of consuming fresh produce irrigated with reclaimed water following the concept of the "Stockholm Framework." The "Stockholm Framework" concept proposes that the tolerable health risks resulting from any water-related exposure (hence also irrigation water use in agriculture) should be the same (Fewtrell and Bartram 2001).

Not all QMRA studies from a water microbiology perspective have compared their risk estimates to a stated acceptable risk level (Asano and others 1992; Finley and others 2009). The formulas of annual risk of infection or illness, or the calculated loss of DALYs pppy can be used to evaluate and quantify the risks associated with the use of certain types of irrigation water or the consumption of certain types of vegetables. But these formulas can also be used in a different approach, such as the translation of a tolerable risk level to operational targets (targets for the irrigation water quality or for the efficiency of implemented risk mitigation strategies). This approach is more useful for establishing operational health-based targets (WHO 2006) and has been used in the report on Australian guidelines for water recycling (NRMMC-EPHC-AHMC 2006) and by Stine and others (2005).

Despite the fact that several papers refer to the tolerable health risk set by the WHO of 10^{-6} DALY loss pppy, the DALY metric is generally not adopted. The reason for this is because the

Table 7–QMRA studies with a water-perspective that used one of the benchmark acceptable risk levels and their outcome.

RA studies with W-background	Outcome	Used benchmark acceptable risk level
Diallo and others (2008); Ferrer and others (2012); Hamilton and others (2006a,2006b); Munoz and others (2010); Navaro and Jimenez (2011); Shuval and others (1997); Tanaka and others (1998); van Ginneken and Oron (2000)	Infection risk pppy	U.S. EPA benchmark "$\leq 10^{-4}$ infection risk pppy"
Petterson and others (2001a)	Likelihood of infection (number of people/ 10 000 exposed)	
Stine and others (2005, 2011)	Maximum concentration of pathogens allowable in water to meet acceptable risk level	
Seidu and others (2013)	Number of days of irrigation cessation required to achieve annual tolerable infection risk	
NRMMC-EPHC-AHMC (2006)	Health based log reduction targets	WHO benchmark "$\leq 10^{-6}$ DALY loss pppy"
Ayuso-Gabella and others (2011); Barker and others (2013)	Annual burden of disease (DALY loss pppy)	
Al-Juaidi and others (20101); Barker-Reid and others (2010); Bastos and others (2008); Lim and Jiang (2013); Mara and others (2007); Seidu and others (2008); Mara and Sleigh (2010b); Pavione and others (2013)	Infection risk pppy	QMRAs that refer to the benchmark "$\leq 10^{-6}$ DALY loss pppy," but used as tolerable risk level a "translated" tolerable infection risk pppy of this initial tolerable risk level of $\leq 10^{-6}$ DALY loss pppy.
Forslund and others (2010, 2012)	Disease risk pppy[a]	For example, a tolerable infection risk of 10^{-3} pppy for rotaviruses and *Cryptosporidium* and 10^{-4} pppy for Campylobacter (WHO (2006)).

[a]Used a maximum permissible annual diarrheal disease risk of 1×10^{-3} pppy, derived from the WHO benchmark 10^{-6} DALY pppy.

DALY metric requires additional information, such as the relationship between infection and illness, the disease burden, and the proportion of the population susceptible to developing disease symptoms following infection. A data gap identified in the selected literature is the absence of country-specific data for the calculation of disease burden (DALYs per case of illness of a certain pathogen). As such, epidemiological data needed for calculation of disease burden or values of disease burden itself are often obtained from other studies/countries and used as a surrogate for the situation under study (NRMMC-EPHC-AHMC 2006; Ayuso-Gabella and others 2011; Drechsel and Seidu 2011; Barker and others 2013). This was also the case for those studies that calculated the tolerable annual illness or infection risk based on the WHO benchmark of acceptable risk (maximum additional burden of disease of 10^{-6} DALY loss pppy; Mara and Sleigh 2010b; Lim and Jiang 2013). Finally, disease burden estimates have not been reported for all pathogens and/or they cannot be easily determined. This is the case, for example, for enteric viruses which cause diverse symptoms ranging from mild to severe (Hamilton and others 2006a).

Arguments for making the current tolerable risk levels less strict are available in the literature (Haas 1996; Mara 2011). Haas (1996) has argued that some key factors used for the initial computation of the 1:10 000 level of acceptable risk may not be accurate. For example, computation of the currently used risk level from the late 1980s appears to have arisen partly because, at that time, the perceived waterborne disease rate was 1 case per 10000 people per year. But more recent assessments of the actual burden of waterborne illnesses appear to be much higher (Haas 1996; Colford and others 2006). As such it may be that an annual risk of infection of 1 in 1000 (or even a less serious risk level) is more appropriate than the current approach (Haas 1996). Mara (2011) advocates for lowering the benchmark of maximal additional burden of disease (a 10^{-6} DALY loss pppy) to 10^{-4} DALY loss pppy, based on critical analysis of the basis from which the current benchmark is derived: U.S. EPA's acceptance of a 70-y lifetime waterborne cancer risk of 10^{-5} per person (Mara 2011).

Several studies selected for this review objected to the use of stringent benchmarks of tolerable risk level. Lim and Jiang (2013) questioned the appropriateness of the U.S. EPA $\leq 10^{-4}$ infection pppy risk benchmark in their efforts to assess sustainable water practices, such as the use of rooftop-harvested rainwater, for unrestricted irrigation of home-grown vegetables. In the context of wastewater irrigation, Mara and others (2007) proposed that a less-stringent tolerable level for the risk of infection is of 10^{-2} pppy (once every 100 y, essentially once in a lifetime; or 1% of the community per year). This revised tolerable risk level was considered by several other studies (Barker-Reid and others 2010; Pavione and others 2013; Seidu and others 2008, 2013).

In food-oriented QMRA studies, benchmarking risk estimates to a tolerable risk target is uncommon, largely because of lack of an agreed-upon set of food safety objectives or public health goals. In this review of QMRAs with solely a food science perspective, there was not even a standardized outcome expression for risk (Table 8). For example, some studies calculated the number of illnesses to be expected from the consumption of a particular item among the population in a specific country/situation (Danyluk and Schaffner 2011; Ottoson and others 2011); others calculated the probability of illness per serving (Domenech and others 2013). The use of tolerable or acceptable risk values continues to be hotly debated in food safety circles, but it should be noted that "acceptability" is not only based on scientific data, but also on social, ethical, and economic considerations, and thus is part of risk management and not of risk assessment (Reij and van Schothorst 2000). This is reflected in the majority of QMRA studies from a food perspective and reflects the purpose of such assessments, which is frequently about comparing potential risk mitigation strategies rather than coming up with specific regulations.

Risk mitigation strategies under consideration in selected QMRA studies and lessons learned

QMRA can be used as a tool to assess the impact of different risk mitigation strategies. Once the "baseline" model is constructed, different scenarios can be evaluated and their relative impact on exposure or illness can be calculated (CAC 1999). The use of sensitivity analysis has also been acknowledged as an appropriate tool

Table 8–Risk outcome of RA with solely a food-background and their used "acceptable risk reference."

RA studies with solely F-background	Outcome	Used acceptable risk level
Mota and others (2009)	Annual risk of infection from exposure to *Cryptosporidium or Giardia* through the consumption of tomatoes, or bell peppers, or cucumbers, or lettuce.	At the end, they mention that the U.S. EPA recommends that drinking water not pose an annual microbial risk of infection greater than 10^{-4}. But did NOT compare their risk estimates with this value.
Carrasco and others (2010)	Mean number of cases of listeriosis per year in Spain because of ready-to-eat lettuce salads, and prevalence and concentration of the pathogen in the food at time of consumption.	A desirable general goal was a level of 100 CFU/g in the product at the time of consumption (as in regulation (CE) No. 2073/2005).
Rodriguez and others (2011)	Estimates on concentration and prevalence of *E. coli* O157:H7 populations in commercially fresh-cut bagged lettuce (an exposure model).	/
Ottoson and others (2011)	The probability of illness (P_{ill}) and number of illnesses per 10000 servings.	Is absent, but is not relevant as the goal was to compare the relative difference because of different risk-mitigation strategies.
Danyluk and Schaffner (2011)	Number of illnesses	/
Ding and others (2013)	Contamination level of lettuce at the time of consumption, probability of listeriosis illness per person per day eating lettuce, annual probability of listeriosis illness for consuming lettuce per person, and annual cases of listeriosis per year in Korea.	Compared contamination level of lettuce with food safety limit of *L. monocytogenes* on fresh produce fixed at 2 log CFU/g.
Domenech and others (2013)	The mean, 5% and 95% percentile of probability of illness at home per person per serving depending on the initial load of lettuce at retail.	To comply with the U.S. Healthy People 2020 initiative which aimed to reduce the rates of listeriosis by 50 percent it was calculated that the probability of illness must be less than 1.32×10^{-8} listeriosis cases per serving to attain this level of protection.
Puerta-Gomez and others (2013)	The probability of infection from a serving of ready-to-eat spinach.	As more than 1% (that is, 10^{-2}) of probability infection is considered unsafe for food processors, this value was used as the tolerance level in this study (= 1.33 $\log_{10}$ CFU/g of sample)

to identify possible risk management options (Carrasco and others 2010; Lim and Jiang 2013), whereas scenario analysis is used to compare mitigation strategies (Rodriguez and others 2011). But still, in decision making it is important to bear in mind that constructing a QMRA will always include a minimum number of assumptions which will contribute to the overall uncertainty and decrease the reliability of conclusions drawn. When possible, validation of a model should be attempted. Interestingly, only one study explicitly stated that the model was validated using experimental values obtained in laboratory settings, which was not included in the model; this was done using Standard Error of Prediction (SEP) method (Rodriguez and others 2011). Some other studies compared the obtained level of infection/illness probability or number of illnesses with the actual situation presented by country-/region-specific disease statistics (Carrasco and others 2010; Ding and others 2013). In this section, risk mitigation strategies that were investigated at the farm level (including selection criteria for irrigation water), at processing, and at the consumer level will be covered. Subsequently, the overall lessons learned from the selected QMRA literature concerning the use of water in the fresh produce supply chain will be discussed.

At the farm level, different (waste)water treatment options were assessed to identify level of treatment necessary for irrigation of produce that is safe for human consumption (Tanaka and others 1998; Munoz and others 2010). Different types and contamination levels of treated wastewater used for irrigation (Navarro and Jimenez 2011; Barker and others 2013; Lim and Jiang 2013), and different national (Ottoson and others 2011) and international (Bastos and others 2008) criteria for irrigation water quality have been evaluated for their impact on food safety. But QMRA has also been used to evaluate the impact of irrigation method (such as drip, furrow, or overhead; Stine and others 2005); identify an appropriate withholding period (Stine and others 2005; Barker-Reid and others 2010; Ottoson and others 2011); investigate the possibility of crop selection/restriction (Stine and others 2005; Bastos and others 2008); explore the use of biosolids having different microbiological contamination levels as soil amendments (Navarro and Jimenez 2011); and the value of microbiological criteria at primary production (Carrasco and others 2010). In such studies, when the risk of different types of crops was compared, lettuce was frequently considered the most hazardous (Hamilton and others 2006a; Mota and others 2009; Lim and Jiang 2013).

At the processing level, the impact of washing and the use of disinfection treatments such as chlorination (Rodriguez and others 2011) and ionizing radiation (Rodriguez and others 2011; Puerta-Gomez and others 2013) have been examined. Implementation of different sampling plans for lot acceptance by testing of final product (Rodriguez and others 2011); reduction in maximum shelf-life indicated on package (Carrasco and others 2010); a change in the packaging atmosphere (Carrasco and others 2010); and the efficacy of cleaning and disinfection procedures (Rodriguez and others 2011) on concentration and prevalence of pathogens or risk of illness have all been evaluated using QMRA. Carrasco and others (2010) also modeled a hypothetical and ideal situation of 100% compliance with regulation (CE) No. 2073/2005, that is, a concentration of *L. monocytogenes* in product at time of consumption of 100 CFU/g, as compared with the baseline model.

At the consumer level, the effect of produce washing and/or disinfection at home (Ottoson and others 2011; Barker and others 2013; Domenech and others 2013); and risk communication strategies to reduce the probability of consumption of RTE lettuce salads by high-risk populations (relative to listeriosis risk; Carrasco and others 2010) have been assessed using QMRA. Sensitivity analysis suggested limiting serving size/consumption rate

(Carrasco and others 2010; Lim and Jiang 2013) and better home storage temperature control as potential mitigations for listeriosis (Carrasco and others 2010). However, the former was not further considered because of the broad known health benefits of fresh produce consumption (Lim and Jiang 2013).

There are some key lessons learned from the selected QMRAs for each phase of the farm-to-fork continuum. At the farm level, *selection of an appropriate water source and/or degree of treatment* for irrigation water is critical. For instance, in a study by Ferrer and others (2012), the use of canal surface water (wastewater) in Thailand for the irrigation of raw vegetables proved to result in a yearly infection risk of 100% for *Giardia* and *Entamoeba*. Also, the use of rooftop-harvested rainwater in the United States for overhead irrigation of home-grown lettuce, cucumber, and tomatoes led to unsatisfactory infection risks when compared to the U.S. EPA risk benchmark (<1:10000 pppy) for *Salmonella* spp. and *Giardia lamblia* (Lim and Jiang 2013). Barker and others (2013) suggested 3 control points for domestic greywater reuse as irrigation water for home-produced fresh produce, being (i) appropriate choice of greywater source (bathroom water preferred above laundry water); (ii) "opting out" of greywater use on days when a household member is ill; (iii) the use of biocides (particularly in laundry water) could reduce microbiological contamination of greywater.

Use of nondisinfected secondary treated reclaimed water for fresh produce irrigation leads to an unacceptable high annual risk of infections (Tanaka and others 1998; Hamilton and others 2006a). Increasing the microbial removal efficiency of wastewater treatment is associated with a reduction in public health risk associated with fresh produce consumption (Al-Juaidi and others 2010; Pavione and others 2013). Therefore, the use of a tertiary treatment step with disinfection was judged necessary to adequately contain infection risks of, for example, enteroviruses when eating fruits and vegetables (Munoz and others 2010).

There is a need to determine the impact of agricultural water on contamination of fresh produce with human pathogens. Empirical data and experience with (historical) procedures for water treatment and water quality (as described by Uyttendaele and others (2015)) are valuable tools to help establish guidelines for safe water and food. Still nowadays the use of microbiological risk assessment to estimate the required log reduction of microbial contamination in water needed to reduce risk to an acceptable level in fresh produce is recommended by WHO (2006). In several QMRA studies, the objective was to set the health-based log reduction target necessary to meet a predetermined tolerable risk (as in NRMMC-EPHC-AHMC 2006). In an Australian context, viruses required the highest (6.1) $\log_{10}$ reduction to meet the health target. This is because of the high concentration of viruses in domestic wastewater, but also because of their low infectious dose compared to bacteria (reduction of 5.0 $\log_{10}$ *Campylobacter* was needed) and the high disease burden compared to protozoa (reduction of 4.8 $\log_{10}$ *Cryptosporidium* was needed; NRMMC-EPHC-AHMC 2006). These findings are consistent with the higher disease risk for viruses relative to other enteric pathogens generally obtained when QMRA was performed for different classes of pathogens (Mara and others 2007; Bastos and others 2008; Pavione and others 2013). However, this 6 $\log_{10}$ reduction required for enteric viruses does not solely have to be obtained using water treatment options, but other nontreatment options (different irrigation practices, implementation of a withholding period, postharvest processing) can also be part of a multibarrier approach (Drechsel and Seidu 2011). Stine and others (2005) used QMRA to help set irrigation water quality standards for enteric bacteria and viruses. When furrow irrigation was used for production of cantaloupe or lettuce (and a worst-case scenario in which produce is harvested and consumed the day after the last irrigation event and maximum exposure is assumed), 2.5 CFU/100 mL of *Salmonella* and 2.5×10^{-5} most probable number per 100 mL of hepatitis A virus would be the maximum concentration allowable in irrigation water to ensure an annual risk of $\leq$1:10000 (Stine and others 2005).

The second important factor at the farm level is the use of appropriate *water application practices*. First, the selection of an appropriate *type of irrigation method* must be considered. Several QMRA studies modeled the impact of different irrigation practices (van Ginneken and Oron 2000; Stine and others 2005; Al-Juaidi and others 2010). Only a minority were actually based on experimental studies (Stine and others 2005). The study by Stine and others (2005) suggested that subsurface drip irrigation reduces the risk of crop contamination compared with furrow irrigation. However, the impact of irrigation method on microbial contamination can be crop specific. For instance, contamination of fresh produce by contact with irrigation water can be dependent on the physical properties of the edible portion of the plant, such as surface texture and the location of the edible portion of the plant in reference to the irrigation water (Stine and others 2005). High-and-low-growing crops represent different soil-effluent-plant contact situations (Bastos and others 2008; Pavione and others 2013). For example, in contrast to cantaloupe and lettuce, no microorganisms (PRD1 phage or *E. coli*) could be detected on bell peppers after subsurface drip or furrow irrigation, which is a typical example of a crop growing relatively high above ground (Stine and others 2005). Comparatively speaking, lettuce poses the highest infection risk (Hamilton and others 2006a; Bastos and others 2008; Mota and others 2009; Lim and Jiang 2013) because of its relatively high water retention rate. Consequently, crop selection has been identified as an effective complementary health hazard control measure (Bastos and others 2008).

Another aspect of water application practice is the selection of a *withholding period*. Generally, a withholding period has a positive influence on risk and has been identified as a risk mitigation strategy (van Ginneken and Oron 2000; Hamilton and others 2006a). However, the magnitude of the influence of the withholding period is dependent on the environmental conditions, the quality of the water used, and the pathogen of interest (Hamilton and others 2006a), as some pathogens have a relatively long survival time in the environment (such as *A. lumbricoides* and protozoa) compared to others (such as *Campylobacter*).

In addition to irrigation, water can also be used for *delivery of pesticides* in the form of a spray, whereby the spray can make direct contact with the edible portion of the plant, serving as a source of pathogen contamination. Hence, pathogen concentrations in water used for pesticide dilution should also be contained in order to comply with acceptable risk levels (Stine and others 2011).

At the processing level, water can be part of a risk reduction strategy, but when no appropriate sanitizer is used, it can contribute to cross-contamination (Holvoet and others 2014). Several studies included washing for risk reduction during processing (Carrasco and others 2010; Puerta-Gomez and others 2013). However, commonly used packinghouse practices (water washing, and liquid sanitization treatments using chlorine) are not adequate to ensure the safety of the produce when initial or cross-contaminated microbial loads are elevated (Puerta-Gomez and others 2013). Generally, washing results in a microbial reduction of about 1

to 2 $\log_{10}$ (Lopez-Galvez and others 2009), remembering that the washing step has also been identified as a possible route of cross-contamination. The model of Danyluk and Schaffner (2011) predicts that a majority of simulated cases of illnesses in the 2006 *E. coli* O157:H7 spinach outbreak arose from leafy greens cross-contaminated during washing. In a study by Rodriguez and others (2011), a noticeable reduction in the number of lettuce bags contaminated with *E. coli* O157:H7 resulting from cross-contamination was obtained when preserving a concentration of 50 to 200 ppm chlorine in the washing bath, compared to the baseline scenario which did not include any intervention step. However, when the initial batch entering the production line was highly contaminated (such as 100 cfu/g), chlorination (200 ppm) was not as effective in reducing cross-contamination compared to the scenario of a lower-contaminated initial batch. Even when all possible interventions were performed (washing with chlorine, irradiation, sampling plans), there remained a small probability of *E. coli* O157:H7 contamination, confirming that zero risk does not exist.

Traditionally, microbiological criteria have been established to improve food safety. However, it has been suggested that these are less effective at managing risk when low levels of contamination are considered, which is the most likely situation under most field and processing conditions (Rodriguez and others 2011). Carrasco and others (2010) suggested that other measures such as the use of a modified atmosphere packaging or reduction of the product's shelf-life may be more effective in reducing the number of listeriosis cases, for example, associated with consumption of ready-to-eat lettuce salads in Spain.

Finally, at the consumer level, several QMRA studies have identified produce washing as an important intervention step for lowering enteric disease risks associated with the consumption of fresh produce (Navarro and others 2009; Ayuso-Gabella and others 2011; Domenech and others 2013). For example, in the QMRA model of Ottoson and others (2011), rinsing for 15 s under running tap water gave rise to an average 6-fold reduction in the risk of illness associated with *E. coli* O157 contaminated lettuce. In an Australian study, washing of lettuce was estimated to reduce the burden of NoV illness because of home-produce lettuce consumption (irrigated with greywater) by about 1.5 to 2 $\log_{10}$ DALYs pppy (Barker and others 2013).

Further perspectives in risk assessment related to use of water and safety of fresh produce

From a public health point of view, the disease syndrome (for example, gastroenteritis or diarrheal disease) may be of greater interest than the specific cause of the disease (bacteria or noroviruses). In this case, a multirisk assessment approach is sometimes undertaken. For example, Diallo and others (2008) developed a risk assessment that included *Cryptosporidium*, *Giardia*, and diarrhegenic *Escherichia coli* to assess the infection risk of diarrhea-related pathogens in a tropical canal network. The choice of pathogens was justified as these were estimated to be the etiological agents responsible for about 47% of diarrhea in Thailand. Unfortunately, the risk for infection with these pathogens by consumption of 100 g of irrigated vegetables was assessed individually and not combined. In the literature outside the scope of this review (de Man and others 2014), there are examples in which health risk because of ingestion of urban floodwater was assessed by determining the risk of infection for a set of waterborne pathogens that can cause gastrointestinal diseases. The overall risk of infection per exposure event was calculated and comprised the risk of infection with *Campylobacter jejuni* and/or *Cryptosporidium* and/or *Giardia* spp., and/or noroviruses, and/or enteroviruses. The use of the DALYs approach for these purposes has also been proposed, as this possesses the flexibility to aggregate all the risks presented by different pathogens to one single DALYs value (Lim and Jiang 2013).

Overall, considerable progress has been made in recent years in understanding transmission of human pathogens in the fresh produce supply chain, and the role of water (Gil and others 2015; Uyttendaele and others 2015; Van Haute and others 2015). Increasingly, scientific literature on this topic is coming available, providing better data for microbial risk assessment. But, apart from water, also the role of food handlers in transmission of food borne pathogens to fresh produce, in particular for viral agents such as norovirus, is being recognized. The role of good hygienic practices has been included in some quantitative microbial risk assessment models, especially those targeting food preparation in retail and institutional food service operations (Mokhtari and Jaykus 2009; Stals and others 2015). This provides opportunities for more elaborate microbial risk assessment models that integrate both the impact of water and breaches of good hygienic practices by food handlers.

To assess the risk on human health because of consumption of vegetables irrigated with (treated) wastewater, ideally both chemical and microbiological risks should be assessed simultaneously and perhaps cumulatively. In a study by Munoz and others (2010), both chemical and microbiological (enteroviruses) risks are evaluated in parallel. The possibility of analyzing these risks from a cumulative point of view using the concept of disability-adjusted life-years (DALYs) was stated, but it was concluded that this was not possible because of lack of available DALY values for many microorganisms as well as for the organic pollutants included in the study.

When evaluating (treated) wastewater as a water source for irrigation and, hence, contamination, intake of more than one produce type would be relevant. This approach can be referred to as multisource exposure calculations. An example of this approach is provided by Pavione and others (2013). These investigators calculated the risk of infection for each of the reference pathogens (rotavirus, *Campylobacter*, *Cryptosporidium*) by consuming low-growing salad crops and high-growing crops. Each of these groups consisted of various kinds of vegetables of which mean consumption data per person per day were available.

Exposure to pathogens (such as those present in wastewater) can occur via multiple pathways. When risk of infection by multiple pathways is estimated as a single-risk outcome, the process can be defined as multipathway risk assessment. An example can be found in a QMRA study done by Seidu and others (2008). These investigators examined the risk of exposure associated with consumption of wastewater-irrigated lettuce, but also the risk from exposure because of both accidental ingestion of irrigation water and contaminated soil. The latter resulted in a combined annual risk of infection from exposure to wastewater and contaminated soil for the farmers. Note that these multihazard, multipathway, and multisource QMRAs are relatively new approaches and will need further development to expand their usefulness.

Quantitative microbial risk assessment can provide an objective and scientific basis for risk management decisions. However, the link from risk assessment to risk management is still challenging as clearly demonstrated by Bichai and Smeets (2013). These authors found that QMRA played a different function in Australia and the Netherlands, despite both countries being considered leaders in the use of QMRA in water regulation. Although the Netherlands placed more emphasis on the value of knowledge gained from the

process of constructing QMRAs, Australia relied more heavily on a strict decision-making value.

Conclusions

The use of QMRA to manage fresh produce safety risk is complicated by a vast number of produce items, production/processing conditions, as well as the lack of supporting data leading to uncertainties or variability in the outcomes. Still, the selected QMRA studies discussed here demonstrate their use in specific situations, in some instances in support of decision-making on the use, quality, and treatment of water used across the fresh produce supply chain. Overall, having analyzed the selected QMRA studies it can be concluded that viruses often resulted in the highest risk estimates and leafy greens were the commodity of greatest concern. With regard to other aspects on the use of water in the fresh produce chain, cultural differences in food preparation, the susceptibility of different populations, and regional variation in the prevalence and concentration of pathogens in (waste) water and environmental conditions means that the results obtained by one QMRA study cannot always be translated to other situations or regions. There are many sources of uncertainty that might arise from inputs to a risk assessment: measurement errors, sampling errors, systematic errors, estimated (using surrogates) or excluded variables, incorrect model forms, and abnormal conditions . The QMRA models are constructed based on the best knowledge and available information (parameters and data) at the time of development. For example, QMRAs that make use of ratios to estimate the concentration of pathogens in water may need to be revised if better data become available. QMRA studies are particularly useful in evaluating different control scenarios, but as the outcomes rely partly on assumptions, results should be interpreted as an indication of the level or degree of safety and not as absolute values. Still, the outcomes of these exercises can be used to guide the risk management in preventing contamination, controlling it, if it occurs, and identifying areas in need of further research or data collection. Overall, the use of QMRA is leading to more flexibility and more tailored guidelines on water treatment and levels of pathogens in irrigation or processing water for the fresh produce in certain regions. Drawbacks are, however, capacity and knowledge to perform the QMRA and the need for data relevant to the specific regions.

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Authors' Contributions

Conception, planning, and interpretation of the paper was done by L.J., P.A., G.M., P.M., L.-A.J., and M.U., followed by organizing, collection of relevant literature, interpretation, and writing performed by A.D.K., L.J., and M.U. and major contributions on rephrasing and writing style made by L.-A.J.

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