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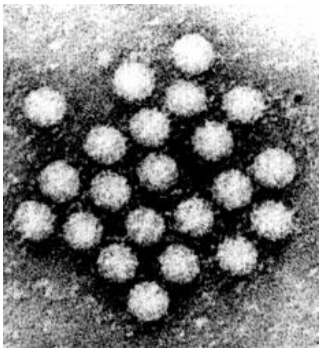
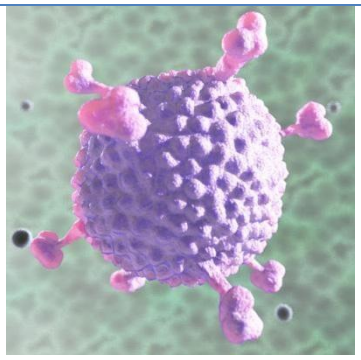
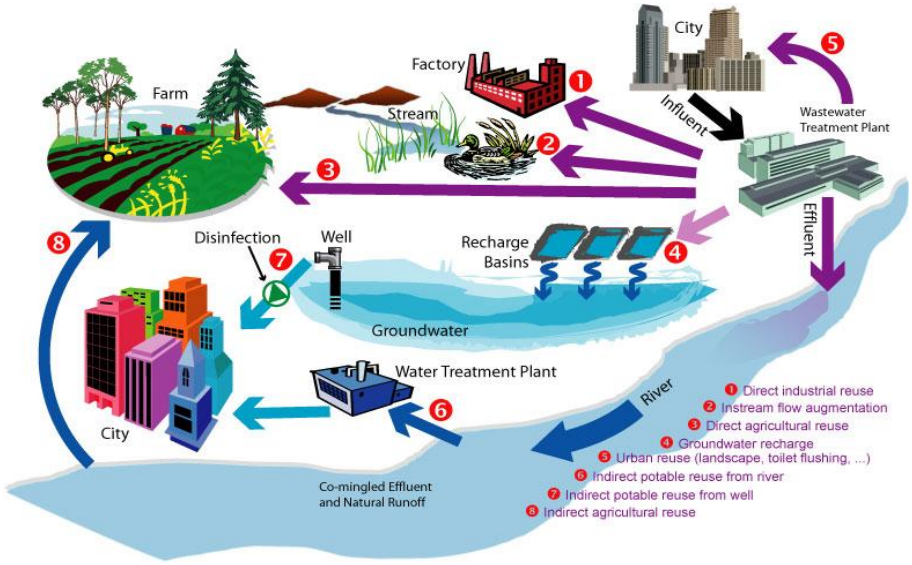
Microbial risks of reclaimed water uses in agriculture: Development of a QMRA model







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Supervisor host institute: Helena Sales-Ortells, PhD and Prof. Dr. G. Medema

Examiner: Dr. Nynke Kramer

Preface

In front of you lies my master thesis on the microbial risk assessment of reclaimed water, which I wrote in co-operation with the Watercycle Research Institute (KWR) at Nieuwegein. At this institution I developed a QMRA model for the risk assessment of noroviruses and adenoviruses in reclaimed water for agricultural purposes.

The aim of this thesis is to review scientific literature for input values, their distribution, and uncertainty for the developed model. The model is developed for concentrations of norovirus and adenovirus in a wastewater treatment plant in Catalonia, Spain.

This thesis consists of a short introduction to the matter of concern (chapter 1). The second chapter focuses on the literature review, which gives background information on each step of the QMRA approach and reviews the input data for the model. Readers interested in detailed information about the development of the model are referred to chapter 3. The following chapter describes the model outcomes and its sensitivity (Chapter 4). Chapter 5 discusses the chosen input data and distribution. This thesis concludes with the results of the developed model and discusses the risks of using reclaimed water for agricultural purposes.

For the opportunity to write my thesis at KWR about this highly relevant topic, I would like to thank Helena Sales Ortells and Prof. Dr. G. Medema. Special thanks to Helena who guided me through a lot of literature, values and helped me understand the fascinating world of modeling. Thanks to Prof. Dr. Medema for his expert advice on distributions and input data for the model. I also would like to thank Dr. Nynke Kramer, for her help with the structure of the thesis, allowing this to be a readable document.

Nieuwegein, 20 december 2013

Wiebke Dürig

Acronyms

Acronym	Meaning
AdV	adenovirus
av.	average
conc.	concentration
CV	canine caliciviruses
DALYS	disability adjusted life years; the sum of years of potential life lost due to premature mortality and the years of productive life lost due to disability
FCV	feline caliciviruses
i.v.	infective virus counts
LCL	lower confidence limit
mJ	millijoule
MNV	murine norovirus
MPN	most probable number
n.a.	not available
nm	nanometer
No.	number
NoV	norovirus
PCR	polymerase chain reaction
pdu/L	polymerase chain reaction detectable units per liter
pfu/L	plaque forming units per liter
pos.	positive
ppm	Parts per million (mg/L)
pppy	per person per year
QMRA	quantitative microbial risk assessment
RH	relative humidity
RNA	ribonucleic acid
RT	reversed transcription
SD	standard deviation
UCL	upper confidence limit
UV	ultraviolet light
WHO	World health organisation
WWTP	wastewater treatment plant

Summary

In arid and semi-arid areas, wastewater is a reliable source of water with valuable nutrients for agriculture. However, treated wastewater might contain pathogenic microorganisms that survived the water treatment. The irrigation of food crops with such water may lead to infections in humans ingesting irrigated vegetables. A wastewater treatment plant in Catalonia, Spain, reuses tertiary effluent for golf course and agricultural irrigation. In this thesis the health risks derived from the reuse of wastewater for agricultural irrigation from the plant in Catalonia are assessed by developing a quantitative microbial risk assessment (QMRA) model. The model input parameters are based on scientific literature. Specifically, the health risks derived from exposure to noroviruses and adenoviruses are assessed. These viruses are investigated due to their high infection rate and resistance in the environment. In addition, these viruses cause diarrheal illnesses following ingestion of contaminated food.

The results show that the average risk of getting ill due to consumption of lettuce, which has been irrigated with tertiary effluent, is higher than for tomatoes. The highest risk was obtained for adenovirus on lettuce, followed by adenovirus on tomatoes, norovirus on lettuce and norovirus on tomatoes. According to the WHO guideline from 2010 a yearly risk of 10^{-6} DALYS is acceptable with regard to ingestion of wastewater irrigated crops [48]. The calculated risks of the analyzed scenarios are within this limit, except for the average annual risk of adenovirus contaminated lettuce.

Since most input values chosen for the model are conservative and may not represent the true value, the calculated risks are likely to be overestimated. Other input values may lead to underestimation of the risks. Irrigation of vegetables, as considered in this model, represents a high-risk reuse activity and may not be indicative of risks associated with other uses.

A failure in the tertiary treatment system would lead to a higher concentration of pathogens in the effluent. The plant in Spain has introduced techniques to prevent too high pathogen concentrations in the tertiary effluent due to failures, allowing the high concentrations for only a limited period of time. The risk during the failure of the system has not been assessed in this study. Nevertheless, it is likely that the daily risks will increase for the time the failure occurs.

It is recommended to use concentrations measured in the plant of investigation as irrigation source for the model- if available. It is also suggested to improve the model by choosing more accurate distributions and input values for a more precise estimation of the risk. This can also be achieved by running more diverse scenarios to evaluate the risk of getting ill due to other pathogens associated with ingestion of other raw vegetables from the investigated farms. Failure scenarios in the plant as well as additional contamination sources should be considered when improving the model.

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1. Introduction

In arid and semi-arid areas, wastewater is a reliable source of water and of valuable nutrients for agriculture. The scarcity of water in arid and semiarid regions has encouraged the use of alternative water sources, such as treated municipal wastewater. Reclaimed water is treated sewage that is reused for beneficial purposes [12]. This process is considered sustainable and water conserved as treated wastewater is a stable water source. However, treated wastewater may contain pathogenic microorganisms that survived the water treatment [6, 10, 46, and 48]. These microorganisms can infect humans coming into contact with the reused water.

To minimize the risk of an infection or illness, the WHO gives recommendations for levels of intestinal nematodes and E. coli in the reclaimed water [49]. Nevertheless, these recommendations do not account for viruses. Therefore, even if the quality of the reused water from a wastewater treatment plant is according to the WHO guidelines, this does not mean that no viruses are shed in the effluent of the treatment plant [49].

In Spain, different wastewater treatment plants reuse their water for diverse purposes such as irrigation of golf courses, food crops, and recreational impoundment. A wastewater treatment plant in Catalonia, Spain, reuses tertiary effluent for golf course and agricultural irrigation. The risk for humans of getting ill through ingestion of the irrigated produces from the farms needs to be assessed.

The aim of this thesis is to develop a quantitative microbial risk assessment (QMRA) model for the ingestion of raw crops irrigated with reclaimed water from a wastewater treatment plant in Catalonia. Potential health risks considered are diseases caused by adenovirus and norovirus. These viruses are investigated due to their high infection rate, resistance in the environment, and because they cause diarrheal illnesses following ingestion of contaminated food.

First of all, a conceptual model is built and the data needed for each step of the model are identified. Then, the literature is reviewed to find quantitative data for each step of the QMRA model. The most appropriate data for the situation in Spain are selected to build a probabilistic model and Monte Carlo analysis is used to derive the results. Finally, a sensitivity analysis is conducted to evaluate effects of the inputs on the model outputs, the annual risk of

adenovirus and norovirus disease. @RISK version 6 was used to build the probabilistic model and to run the sensitivity analysis.

1.1 QMRA

The model is developed based on the quantitative microbial risk assessment (QMRA) approach, which is a transparent, science-based approach that allows the risk manager to use the best available scientific evidence as basis for risk management decisions [47]. This approach is derived from the chemical risk assessment paradigm, which encompasses four basic elements:

1. Hazard identification
2. Exposure assessment
3. Effect assessment (dose-response relationship)
4. Risk characterization

To conduct a QMRA each of the four steps need to be outlined and described in detail. In the first step, a good description of the source to fork pathway and the hazard(s) are necessary. In the exposure assessment part, the exposure route under investigation is described and the hazard is quantitatively assessed. The amount of pathogens at each point in the system is determined and an exposure model is derived from this information. The amount of pathogens can be assessed either via measurements at the source itself or, when that is not possible, reviewing relevant scientific literature. The end point of this step is the estimation of the dose of microorganisms that the consumer is exposed to. The effect assessment determines the health outcome associated with the level of exposure to waterborne pathogens (dose-response models). In addition, the susceptible population is described. In the last step of the QMRA approach, information obtained in the exposure assessment and the effect assessment is integrated to obtain a risk estimate. This risk estimate can be used by risk managers to initiate adequate measures.

In this thesis, the QMRA principle is used because it allows estimating very low levels of risk of infection and/or disease, and because it is a low-cost method for predicting risk of infection and/or diseases. Low levels of risk can be estimated with QMRA as this is a mathematical method which does not deal with detection limits like laboratory analysis do. It is a low-cost method because no laboratory equipment is required. Scientific literature is reviewed to develop an adequate model for evaluating the health risks derived from consumption to raw

vegetables irrigated with reclaimed water from a tertiary wastewater treatment plant in Catalonia, Spain. In particular, the risk of norovirus and adenovirus infection by consumption of tomatoes and lettuce is investigated.

2. Literature Review

In this chapter, relevant literature of each step of the QRMA methodology is reviewed. The hazard is described in detail, the exposure is assessed via quantitative data found in the literature, dose-response models for the different viruses are discussed, and the risk outcome is characterized.

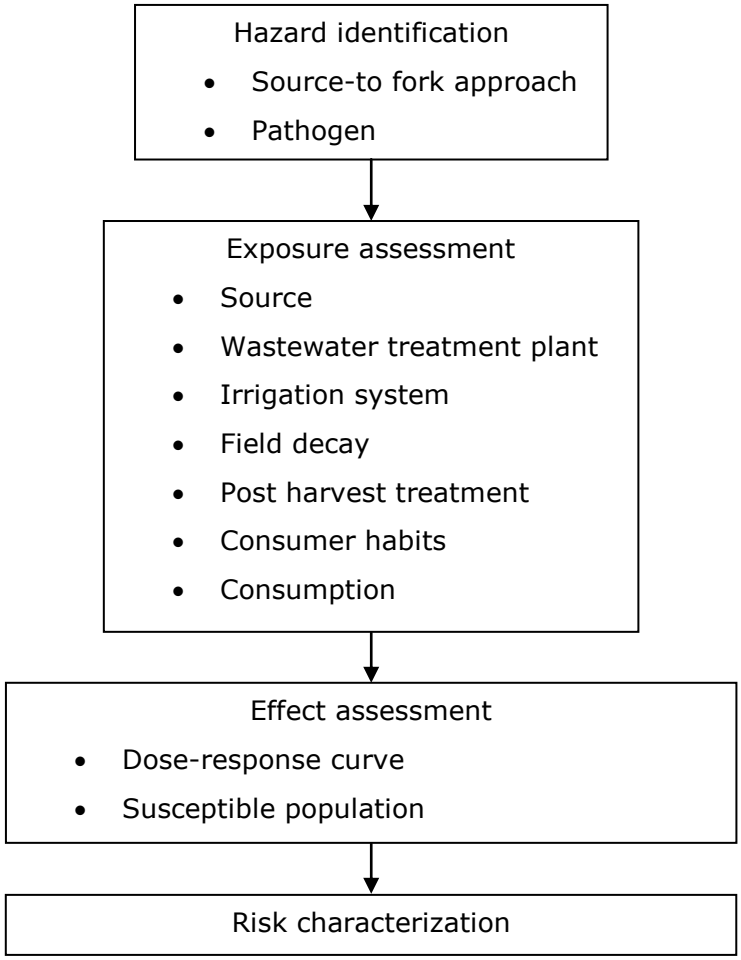

Figure 1: QMRA flow chart

2.1 Hazard Identification

The first step of the QMRA approach focuses on the hazard identification (Figure 1). Hazards, which are possible sources of danger, are identified by characterizing the system under investigation in detail and reviewing literature on the enteric pathogens of interest. In the following subchapters, the system is described from source to ingestion and the different pathogens under investigation are characterized.

2.1.1 Source-to-fork approach

The plant under investigation is located in a semi-arid location in Catalonia, Spain. It receives urban sewage and uses primary, secondary, and tertiary treatment to reduce the concentration of pathogenic bacteria and viruses in the water.

The first treatment comprises flocculation and iron chlorination. During flocculation, clarifying agents are added to produce flakes of colloids in the suspension. With iron chlorination most disease-causing bacteria are removed. Active sludge is used in the secondary treatment. By using air and biological flocculation composed of bacteria and protozoa, carbonaceous biological matter and nitrogenous matter are oxidized and phosphates are removed. Only a portion of the secondary effluent is treated by the tertiary plant, the remainder is discharged into a nearby river. The tertiary treatment consists of sand filtration (20 cm) followed by UV treatment (250 nm) and sodium hypochlorination (3 to 6 ppm). The final chlorine concentration in the effluent is aimed to be between 1 and 3 ppm. The purpose of the UV treatment is to decrease the concentration of sporulated bacteria, which form a single spore within the cell, and viruses. The chlorine treatment, applied in a cascade, aims to obtain zero coliforms in the effluent. Coliforms are commonly used bacterial indicators of sanitary quality of foods and water.

The tertiary effluent is reused by two golf courses and farmers in the surrounding areas. As the focus of this thesis lies on the exposure route of wastewater-irrigated food crop consumption, the farming habits are further described. Approximately 350 m^3 of tertiary effluent per year are used by the farmers. The farms are located approximately 500 m from the wastewater treatment plant.

Irrigation of the fields occurs only on dry days and unexceptionally via the tertiary effluent from the wastewater treatment plant. The water is not stored, but is pumped directly from the plant via underground pipes. The effluent that is not used is discharged into a nearby river. Different kinds of lettuce, basil, parsley, mint, pumpkins, beans, cabbage, broccoli, cauliflower, zucchini and tomatoes are cultivated. Also cherry, fig and plum trees are positioned on the corner of the fields.

Crops are irrigated in the afternoon via spray irrigation, drip irrigation or flooding. Each field is irrigated every second day, but the irrigation system is used every day. Sprinklers and tubes are repositioned each day for another field to be irrigated and reconnected by the farmers, who do not wear gloves during these tasks. Also, the farmers do maintenance work without wearing gloves. Therefore, the farmers are exposed to pathogens in the water system, not only through ingestion and inhalation of droplets formed during spray irrigation, but also through hand-to-mouth contact. However, this risk assessment focuses on the consumers of raw vegetables from these farms rather than occupational exposure.

Spray irrigation results in aerosolization of water pathogens that are likely to deposit on crops surfaces. The pathogens can cling to the surface of the crops. In addition to spray irrigation, flooding and dripping irrigation can also result in virus internalization by the plants. In these cases the pathogens can be taken up by the plants via the roots or via surface cuts on the plant.

There is an elapsed time between the last irrigation and harvesting up to two days. As it is not common to irrigate the produce on the same day of harvest, the elapsed time between last irrigation and harvesting is most likely between 12 and 36 hours. Products, harvested on the morning before the market, are stored in a cold chamber (at 4-7°C). Those harvested the evening before are stored in a concrete house located in the field. Finally, crops harvested the same day of the market are transported directly to the selling point.

The market in which the produce is sold lasts from 7 am to 1:30 pm. The farmers from the wastewater-irrigated field sell their vegetables under an awning. Since the market is an open market, temperatures can rise up to more than 30° Celsius in summer. Sunlight and high temperatures can inactivate viruses and bacteria. However, while bacteria can multiply on and in the crops, pathogenic viruses are not able to multiply outside a human host, so no increase in virus concentration can occur.

Customers, but also the farmers themselves, eat the vegetables raw or cooked. Because cooking temperatures inactivate viruses, the focus of this exposure assessment is on vegetables that are most likely eaten raw, specifically lettuce and tomatoes. Consumers handle their vegetables differently. Some will store the crops in the fridge, while others leave them outside the fridge. Some consumers wash their vegetables carefully whereas others do not. Additionally,

the elapsed time between irrigation and consumption varies from person to person. Differences in handling and 'farm-to-fork' time all contribute to differences in the potential amount of pathogens ingested by the consumer, and so does the amount of pathogens ingested. These differences are all to be considered in an exposure assessment. In the model development consumer habits and elapse time are generalized.

Picture 1 Sprinkler system, tomato and lettuce field near the investigated treatment plant.

2.1.2 Pathogens

In this part of the hazard identification, microorganisms within the system boundaries that could cause human illness and the type of illnesses are described. Ideally, QMRA focuses on a suite of "index pathogens" that cover a range of health risks [47]. Viruses are significantly smaller than bacteria, which can lead to elude filtration barriers designed to remove bacteria [18]. Noroviruses and adenoviruses are chosen as the microbial hazard to assess and model, due to their stability in the aquatic environment, their high infectious rate, and because these pathogens are the most common cause of community gastrointestinal illnesses [37]. Their characteristics, distribution and environmental stability of noroviruses and adenoviruses are described below.

2.1.2.1 Noroviruses

Noroviruses belong to the Caliciviridae family and can be divided into five genogroups [22, 27]. Genogroup I and II are mostly associated with gastrointestinal illnesses in humans [33]. Calicivirus particles are 27-30 nm in diameter, contain single stranded RNA, present an amorphous surface with no envelope, and have a capsid. Noroviruses are obligate intracellular parasites and they are species specific which means that no multiplication occurs outside the living host cell. To date, human pathogenic noroviruses cannot be cultured in any known human cell line. Therefore infectivity research is done with surrogates like murine norovirus, feline calicivirus and canine calicivirus [34].

Noroviruses are neither inactivated at pH 3 nor by ether or chloroform. 30 minutes exposure to 60°C does not inactivate noroviruses either [39]. However, they are inactivated by chlorine at concentration >10 mg/L, although not by free residual chlorine at 0.5-1 ppm [27].

Noroviruses have only recently been identified in different aquatic samples. The virus is found in the Netherlands, Ireland, Japan, and Spain, and among others [22, 33, 19, and 23]. In Spain, among children younger than 5 years old, 47% tested positive for norovirus infection [27]. The virus has also been detected on surfaces used in food preparation. Food served uncooked or handled after cooking is a potential hazard for Norovirus infections. Norovirus concentrations on/in wastewater irrigated vegetables are unknown, but strongly dependent on the level of human faecal contamination in the source water. Concentrations measured in secondary effluent will be reported later in this thesis.

Transmission of noroviruses is mainly direct person to person by the faecal-oral route or by the ingestion of particles of vomit. Aerolization from vomit is a source of infection as well. The viruses cause diarrhea and vomiting after an incubation time of 24-48 hours [27]. Virus replication probably takes place in the mucosal epithelium of the small intestine resulting in damage to the epithelium (enterocytes) and flatting of the villi. Norovirus gastroenteritis occurs in all ages and in sporadic and epidemic disease patterns. However, children less than 5 years old are more susceptible to develop the disease than adults [27].

There is no specific treatment and the symptoms are rarely sufficiently severe to require rehydration. Mortality is rare and has occurred mainly in

people with pre-existing conditions and the elderly. Seropositivity does not affect the susceptibility of re-infection, which makes the virus highly infectious.

2.1.2.2 Adenoviruses

Adenoviridae are a family of viruses that infect both humans and animals. The viruses are 70-100 nm in diameter, have an icosahedral structure containing double stranded DNA, and do not possess a membrane. To date, 52 serotypes of human adenoviruses are recognized and divided in 7 subgroups, designated from A to G, on the basis of their ability to agglutinate red blood cells [48, 18].

Their lack of membrane gives adenoviruses the ability to resist ether and chloroform, but they are inactivated by chlorine, which is also used in wastewater treatment [39]. Adenoviruses are more resistant to the action of UV light than enteroviruses [15, 40]. The viruses are stable in a range of pH from 3 to 10 [10]. Soil in arid regions often has a pH of 7 to 9 [50], meaning that adenoviruses are stable in environmental soil. Furthermore, the viruses are resistant to intestinal enzymes and replicate in the epithelial cells of the intestine. The virus is an obligate intracellular parasite, therefore is not able to replicate in the environment.

Puig et al. (1994) and Pina et al. (1998) detected adenoviruses in sewage, polluted water, and shellfish throughout the year in most samples investigated [32, 30]. The exact levels will are noted in chapter 2.2.1. The virus was also found in California, Australia and Japan [17, 13, and 19]. Bofill-Mas et al. (2006) detected the virus in secondary effluent of a treatment plant in Catalonia, Spain [3]. Adenoviruses are associated with failure of chlorination system in wastewater treatment plants.

Human exposure to adenoviruses occurs via dermal contact, inhalation of aerosolized particles, or ingestion. Adenoviruses infect the respiratory tract, the eye and the gastrointestinal tract [18]. The virus is associated with Bordetella pertussis infection and follicular conjunctivitis. It is usually a mild, short infection from which there is a full recovery. Shipyard eye is a more aggressive disease caused by Adenovirus.

Generally, only virus types 40 and 41 from subgroup F infect the gastrointestinal tract. Incubation periods vary according to the serotypes and the site of infection. Respiratory illness and gastrointestinal disease can occur after a few days. Serotypes 40 and 41 are transmitted via faecal-oral spread. Other

serotypes are spread by aerosols of respiratory droplets. There is no specific treatment for enteric adenovirus infection. However, immunity to group F (enteric) viruses appears to be life-long as the majority of reported infections occur in very young children [18].

2.2 Exposure assessment

The exposure of consumers ingesting raw vegetables irrigated with reclaimed water is estimated quantitatively using published data from scientific literature. First of all, relevant literature on the concentration of noroviruses and adenoviruses at the source is reviewed. Because no information has been found on Adenovirus and Norovirus concentration in tertiary effluent, data on secondary effluent is used, and log reductions due to tertiary treatment based on literature information is applied. The transmission of viruses to the crops is estimated depending on irrigation method, and the survival on crops surface and inside the crops is considered. Virus load might also be reduced due to post-harvest manipulation of crops, such as peeling or rinsing with water. Finally, the consumption of vegetables (amount and frequency) is assessed using Spanish survey data. All gathered relevant information is combined to derive a microbial dose, which will be used to estimate the health risks.

The entire exposure pathway considered in this thesis is depicted in a flow chart in Figure 2. Table 1 lists relevant questions, which need to be considered to assess the adequate exposure for consumers of wastewater-irrigated lettuce and tomatoes. These vegetables are predominantly eaten raw in salad and they retain a relatively large volume of water on the surface of the plant, thus conferring greater potential for transfer of pathogens from irrigation water.

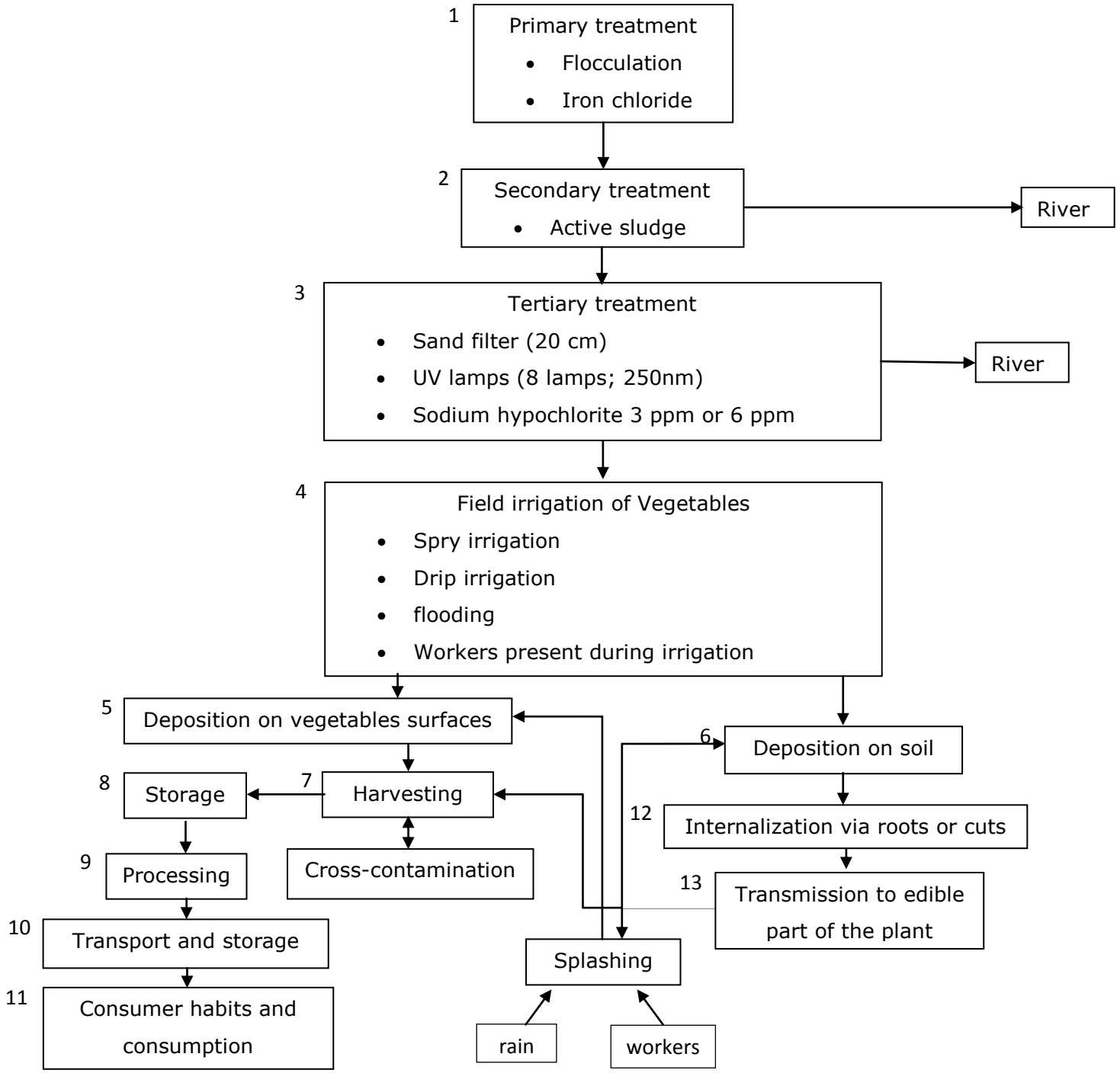

Figure 2: Exposure flow chart of the considered pathway of this thesis. The numbers next to the boxes correspond with the questions in Table 1.

Table 1 Relevant questions for conducting the exposure assessment for the pathway described in the flow chart in Figure 1.

Box	Questions
1	How and which pathogens are eliminated? What happens during failure? How many pathogens are shed in the primary effluent?
2	How and which pathogens are eliminated? What happens during failure? How many pathogens are shed in the secondary effluent?
3	How and which pathogens are eliminated? What happens during failure? What is the end concentration of the pathogens in the 3rd effluent?
4	What is the volume of water used? What is the frequency of irrigation? Which method is used for the irrigation? What kinds of vegetables are irrigated? When does the irrigation take place?
5	How much water is deposited on the vegetable surfaces? What is the relative humidity of the environment? What is the UV intensity of sunlight? What is the temperature in the field? How do these weather parameters affect virus survival?
6	How much water is deposited on the soil? What is the pH of the soil? What is the relative humidity of the environment? What is the UV intensity of sunlight?
7	What is the period of time between the last irrigation event and harvesting? Are the vegetables harvested by hand or via a machine? Do the workers wear gloves during harvest?
8	Are the vegetables stored prior selling or consumption? What are the storage conditions?
9	Do the workers wear gloves during maintenance work? Are the outer leaves of e.g. lettuce cut off? Are the vegetables washed before sale? Are the vegetables disinfected before sale? Are the vegetables peeled before sale?
10	How much vegetables are transported to the market after harvest? How often are the vegetables transported to the market? Are the vegetables exposed to sunlight or high temperatures during sale?
11	How many people buy their vegetables from that market? How many consumers eat raw vegetables? How many consumers wash their vegetables before raw consumption? Do consumers peel their vegetables before raw consumption? How often do the clients consume raw vegetables from the market?
12	Do the crops absorb pathogens via the roots? How many pathogens internalize via surface cuts? How many pathogens internalize via the roots?
13	How many pathogens are transmitted to edible parts of the plant via the roots and surface cuts?

2.2.1 Pathogen occurrence in source water

Waterborne enteric viruses may be present in the effluent of a wastewater treatment plant due to the presence of human excreta within the influent. The density of certain viruses is dependent upon the disease occurrence in the population of the catchment area. The concentration is expected to vary both over time and between pathogen groups because the prevalence of each disease is variable throughout the year.

A summary of norovirus concentrations found in wastewater and effluent is given in Table 2. These data are from studies performing quantitative surveys of noroviruses on wastewater samples collected from different plants all over the world, and from sewage-impacted river water. Studies predominately report more than 88% of the samples positive for norovirus. Most papers document presence/absence data of viruses in treated sewage, however quantitative data are necessary for a solid QMRA.

Lodder and Husman (2005) reported the concentration of norovirus in surface water of Dutch Rivers (Waal and Maas), as well as raw and treated wastewater samples in the winter months [22]. They found an average concentration of 3,5 pdu/ml in treated wastewater (Table 2). Van den Berg et al. (2005) conducted a one-year monthly study in two wastewater treatment plants in the Netherlands [42]. They detected concentrations of 0,57 and 0,97 pdu/mL in treated sewage water. Katayama et al. (2008) investigated six plants in Japan during a one-year monthly survey and found that the highest concentration of norovirus genotype I and II in secondary effluent occurred during the winter months (December-February) [19]. Other studies, also found higher concentrations of Norovirus in winter compared to summer months [33].

The farmers in Catalonia use the treated wastewater only in the dry summer months for irrigation. Therefore, the reported concentrations of norovirus in summer by Katayama et al. (2008) are the most relevant data for the model development [19]. The authors detected average concentrations of 0,087 RT-PCR units/ml and 0,047 RT-PCR units/ml for norovirus genotype I and II, respectively, in secondary effluent after chlorination during the summer months.

Pina et al. (1998) and Puig et al. (1994) reported that 14 of 15 and 16 of 16, respectively, raw domestic sewage water samples, were positive for adenovirus [30, 32]. A summary of concentrations found for adenovirus is given in Table 3.

Already in 1981, Irving and Smith conducted a one-year survey of adenoviruses from secondary effluent in Australia [17]. They detected an average concentration of 0,3 infectious units/ml after chlorination. He and Jiang (2005) found average concentrations of 850 and 1400 viral genomic copies/mL in secondary effluent after chlorination in summer and winter, respectively [13]. Bofill-Mas et al. (2006) measured an average concentration of 4,69 RT-PCR units/ml in a secondary treatment plant in Catalonia (Spain) in November 2004 and 2005 [3]. This concentration is in the same order as that found by Katayama et al. (2008) in Japan (7 RT-PCR units/ml) [19]. In the reviewed studies, between 71,4 and 100% of the samples were positive for adenovirus (Table 3).

Table 2 Summary of source water norovirus concentrations obtained from the literature. G= genotype, n.a. = not available.

	No. of samples	No. of pos. samples	Concentration	Method	Source
av. conc. in river Maas in the Netherlands in winter	5	5	0,2 PDU/mL	RT-PCR	[22]
av. conc. river Waal in the Netherlands in winter	5	5	2 PDU/mL	RT-PCR	[22]
av. conc. raw sewage in the Netherlands in winter	5	5	200 PDU/mL	RT-PCR	[22]
av. conc. treated sewage in the Netherlands in winter	5	5	3,5 PDU/mL	RT-PCR	[22]
conc. in treated effluent in the Netherlands (plant 1) Oct 2000-Oct 2001	14	13	0,57 pdu/mL (mean) <0,01 to 10 pdu/mL (range)	RT-PCR	[42]
conc. In treated effluent in the Netherlands (plant 2) Oct 2000-Oct 2001	14	13	0,97 pdu/mL (average) <0,01 to 10 pdu/mL (range)	RT-PCR	[42]
winter average of a year in Japan (2nd effluent)	71	65+63 (GI+GII)	2,6-2,9 RT-PCR units/ml	RT-PCR	[19]
summer average of a year in Japan (2nd effluent)	n.a.	n.a.	0,047-0,087 RT-PCR units/mL	RT-PCR	[19]
av. conc. GI influent in Ireland ± SD (range) Jan - March 2010	13	n.a.	85,11 ± 0,028 (12,02-575,44) genome copies/mL	real-time RT- PCR	[33]
av. conc. GII influent in Ireland ± SD (range) Jan - March 2010	13	n.a.	380,19 ± 0,024 (95,499-2187,76) genome copies/mL	real-time RT- PCR	[33]
av. conc. GI 2nd effluent in Ireland ± SD (range) Jan - March 2010	13	n.a.	10,23 ± 0,0427 (0,53-114,82) genome copies/mL	real-time RT- PCR	[33]
av. conc. GII 2nd effluent in Ireland ± SD (range) Jan - March 2010	13	n.a.	27,54 ± 0,0295 (2,19-97,72) genome copies/mL	real-time RT- PCR	[33]

Table 3 Summary of source water adenovirus concentrations obtained from the literature. n.a. = not available.

	No. Samples	No. pos. Samples	Concentration	Method	Source
whole year av. conc. raw sewage in Australia	26	25	1,95 infectious units/mL	cell culture with MPN formula	[17]
whole year av. conc. primary effluent in Australia	26	23	1,35 infectious units/mL	cell culture with MPN formula	[17]
whole year av. conc. secondary effluent in Australia	26	23	0,25 (0-0,6) infectious units/mL	cell culture with MPN formula	[17]
whole year av. conc. secondary effluent after chlorination in Australia	7	5	0,3 (0-1,15) Infectious units/mL	cell culture with MPN formula	[17]
Adenovirus type 2 and 12 in raw domestic sewage	16	16	n.a.	nested PCR	[32]
Human adenovirus in raw domestic sewage	15	14	n.a.	Nested PCR	[30]
Human adenovirus in primary treated effluent	3	2	n.a	Nested PCR	[30]
av. conc. primary effluent in California in summer	n.a.	n.a	680 viral genomic copies/mL	real-time PCR method	[13]
av. conc. secondary effluent in California in summer	n.a.	n.a	610 viral genomic copies/mL	real-time PCR method	[13]
av. conc. secondary effluent with chlorine in California in summer	n.a.	n.a	850 viral genomic copies/mL	real-time PCR method	[13]
av. conc. primary effluent in California in winter	n.a.	n.a	740 viral genomic copies/mL	real-time PCR method	[13]
av. conc. secondary effluent with chlorine in California in winter	n.a.	n.a	1400 viral genomic copies/mL	real-time PCR method	[13]
av. conc. secondary effluent in November 2004+2005 (range & sigma) Assay1	7	n.a.	4,69 RT-PCR units/mL (1,78-9,03 & 4.69)	real-time quantitative PCR	[3]
average of a year in Japan 2nd effluent after chlorination (range)	71	71	7 RT-PCR units/ml (1.38-33.11 RT-PCR units/ml)	RT-PCR method	[19]

2.2.2 Wastewater treatment

Wastewater treatment plants are designed to reduce the concentration of pathogenic bacteria and viruses in the water. However, viruses may be resistant to certain techniques used in the plants and, therefore undesired concentrations of pathogenic viruses may be present in the effluent.

The tertiary treatment used in the wastewater treatment plant in Catalonia consists of UV radiation, and chlorination. The UV radiation system of the plant in Catalonia consists of 8 UV lamps disposed perpendicularly to the water flow. The water is pumped with low pressure (~300m^3 h^{-1}) through closed pipes. The objective of this treatment is a fecal coliform reduction of about 4-4.8 log units [personal communication with the WWTP manager]. For controlling the efficacy of the system, sulfate reducing Clostridia, transmittance, and turbidity are measured. The elimination of the viruses through chlorination and UV radiation techniques is discussed below.

Hijnen et al. (2006) reviewed the inactivation efficiency of UV radiation for pathogens in water [15]. According to them, first-order kinetic decay can be applied to describe this inactivation. The authors concluded that adenovirus is more UV-resistant than calicivirus. The same was observed by Thurston-Enriquez et al. (2003b) [40]. The latter discovered that low-pressure UV required to achieve 99% inactivation of feline caliciviruses (FCV) and adenovirus genotype 40 would comprise 16 and 109 mJ/cm^2, respectively [40].

Gerba et al. (2002) found that 119mW/cm^2 is needed for a 3-log reduction of adenovirus type 2 [11]. Thompson et al. (2003) found that a 4-log_{10} reduction was achieved with 168 and 170 mW–s/cm^2 for adenovirus type 15 and 2, respectively, in tertiary effluent [38]. UV doses commonly applied for wastewater treatment range between 30 and 40 mJ/cm^2. This would indicate that adenovirus is not effectively eliminated by UV treatment. Hijnen et al. (2006) argued that the persistence of adenoviruses might be due to the double-stranded genome and complex capside of the viruses [15].

For norovirus surrogates on the other hand, Duizer et al. (2004) found a 2 log inactivation at 21 and 22 mJ/cm^2 for canine calicivirus and feline calicivirus, respectively [9]. They estimated a 3 log reduction after exposure to 34 mJ/cm^2 for both virus types. However, Duizer's study measured the UV inactivation at 0°C which is far below the ambient temperatures expected in Catalonia [9].

Therefore, the reduction found by Thurston-Enriquez et al. (2003b) for FCV is used in the model development [40].

The purpose of the chlorine treatment is to obtain zero coliforms in the effluent. After the UV treatment, the water is mixed with hypochlorite in a cascade. The cascade ensures a good mixing for the redox system. Turbulence and redox are monitored online, but the data is not preserved. The chlorination aims to achieve a 1-3ppm free chlorine concentration after 15 minutes.

Sodium hypochlorite was found ineffective (<1 log inactivation) up to concentrations of 30 ppm free chlorine for norovirus surrogates by Duizer et al. (2004) [9]. Even an increase in exposure time did not result in a significant increase in effectiveness. Thurston-Enriquez et al. (2003a) also investigated the susceptibility of norovirus and adenovirus to chlorine [39]. The efficiency of chlorination is described by the C_t value, which is determined by

$$C_t = \text{contact time} \times \text{free Chlorine concentration} \qquad \text{Equation 1}$$

Thurston-Enriquez et al. (2003a) determined the C_t value for 2-, 3-, and 4-log inactivation under different circumstances [39]. Although the data from Thurston-Enriquez and co-workers are on buffered-demand free water and treated groundwater, they concluded that viral inactivation rates were higher at pH 6 than 8 and higher at 15°C than at 5°C [39]. This accounts for norovirus as well as for adenovirus. They found that adenovirus 40 is reduced by 3- and 4-logs with a C_t-value of 9.69 and 36.09 at pH 8-8.2 and 15°C, respectively. The treatment plant in Catalonia operates at C_t values between 15 and 45. Page et al. (2009) developed a chlorine reduction model for adenovirus type 2 [26]. They applied the data from Thurston-Enriquez et al. (2003a) to their model. The model over predicted the level of adenovirus type 40. This indicates that serotypes of adenovirus are inactivated to different extend by chlorine.

The initial concentration of norovirus used in the model of this thesis is detected after chlorination. Therefore further literature review on chlorination inactivation of norovirus is not needed as chlorination will not occur a second time in the treatment plant.

2.2.3 Irrigation, clinging to surface and internalization

After the wastewater is treated in the plant, it is directly used to irrigate the vegetables on the fields. The crop fields are located at few meters of distance

from the plant, and the farmers do not have a deposit for storing the water, since they can use it directly from the plant. Therefore, phenomena related to virus inactivation occurring during storage and transport of water to the fields is considered negligible.

The tomatoes are irrigated via dripping irrigation and the lettuce is sprinkled via overhead irrigation. Different irrigation practices also differently impact the water deposition on crops' surfaces. Surface drip irrigation can reduce the contamination of produce compared to overhead irrigation [25]. The amount of water clinging to the surface of vegetables may also vary between produce, due to their different surface structure.

Asano and Sakaji (1990) assumed that approximately 1 mL of irrigation water remains on the surface of consumed crops [45]. This value, however, is not based on any scientific measurement. Unlike Asano and Sakaji (1990), Shuval et al. (1997) estimated the volume of water trapped on the surface of lettuces and cucumbers in a laboratory assay [45, 35]. They measured the weight of 12 lettuces and 26 cucumbers before and after fully submersing them in water. The amount of wastewater of varying microbial qualities that would cling to the crops' surface was 0,0036ml g^{-1} for cucumbers, and 0,108mL g^{-1} for long leaf lettuces. These values have been used as point estimates in previous QMRAs [35] and as a normal probability density function with sigma 0.019 [43, 12, and 2]. Since no further studies have been found investigating the amount clinging to the surface of lettuce and tomatoes, the amounts determined by Shuval et al. (1997) are used for the development of the model in this study [35].

It is likely that less irrigation water clings to the surface of tomatoes because they are irrigated via drip irrigation and because of their smooth surface. Drip irrigation provides water on the roots to the plant and smooth surfaces lead to less adhesion of water on the crop surface. However, some aerosols may deposit from the lettuces' sprinklers that are located nearby. Also, insects can transport small amounts of contaminated water, and the water on the soil can be splashed by workers or by rain, and deposit of the tomatoes' surface [1]. Amounts on the above mentioned factors are not available. Therefore, the lower estimated 0,0036ml g^{-1} by Shuval et (1997) for cucumbers is used in the model development for tomatoes [35]. If the edible part of the crop grows in or near the soil, it is more likely to become contaminated than a fruit in the aerial parts

of a plant [11]. Lettuce grows near the root system, whereas tomatoes grow more in the shoot system.

As depicted in the flowchart in Figure 2, deposition on vegetables surfaces is not the only exposure route. Also deposition on the soil followed by internalization via roots uptake or surface cuts, and transport to edible parts of the plant, is a potential route of exposure. Virus removal by soil is believed to occur largely due to adsorption, in contrast with that of bacteria, which are removed by a combination of filtration, sedimentation and adsorption mechanisms [25]. Once viruses are internalized in the plant it would be significantly more challenging to eliminate them by traditional sanitation. The feasibility of internalization of human enteric viruses by plants is supported by the ability of plants to internalize their own viral pathogens, which can be taken up from soil and water [8]. In theory, the efficiency of a smaller pathogen, like viruses, to enter and disseminate in plants would be higher than for bigger pathogens, like bacteria [8]. However, some bacteria have self-motile capacity and are also able to internalize via the roots of the plant. Uptake through internalization is a plant-pathogen specific interaction and presence of internalized pathogens in roots of plants does not directly correlate with internalized pathogens in the edible tissue of the crops [16].

Several researchers investigated the ability of norovirus to internalize in the leaves of lettuce, obtaining different results. Urbanucci et al. (2009) detected canine calicivirus RNA in aerial tissue of lettuce grown both hydroponically and in soil [41]. However, they did not detect viral RNA from the human norovirus genogroup II strain within the inner tissue of the lettuce [41]. On the other hand, Wei et al. (2011) found that less than 2 log of infectious murine norovirus could be detected in leaf samples from days 1 to 5 when the roots were challenged with a high level of the virus ($5x10^8$ pfu/ml) [44]. Most recently, DiCaprio et al. (2012) also found a high level of human norovirus RNA in the leaf tissue of lettuce on the first day of exposure [8].

Several factors may be responsible for this apparent discrepancy in outcomes. One possibility is variation in the experimental conditions between the studies, such as environmental growth conditions (temperature and relative humidity), the type of lettuce tested, the viral strain, and the amount of viral inoculums. DiCaprio et al. (2012) and Wei et al (2011) used romaine lettuce in their study, whereas Rapid lettuce (L. sativa var. crispa) was used in Urbanucci's

study [8, 44, and 41]. The first two studies also grew their lettuce under greenhouse conditions, which does not apply for the situation in Catalonia. All studies performed their experiments in soil as well as hydroponically. Viruses attached to soil particles are less likely to be taken up via internalization than those in hydroponic media [1].

Wei et al. (2011) have shown that lettuce grown under conditions of 99% relative humidity (RH), showed a significantly lower frequency of virus internalization than lettuce grown in a chamber with 70% RH [44]. In the latter case the lettuce had a 10-fold-higher transpiration rate. The RH in Catalonia varies between 69 and 75% [51]. Therefore, it is likely that plants have a high transpiration rate and more pathogens internalize than for produce grown in countries like India or Philippines.

Alum et al. (2011) investigated the internalization of adenovirus type 40 via surface and subsurface drip irrigation of tomatoes and cucumbers [1]. They did not recover any adenoviruses from tomatoes and cucumbers irrigated via surface drip irrigation with adenovirus contaminated water. However, their experiments were performed under greenhouse conditions. It may be possible that adenovirus under the conditions in Spain internalizes into lettuces and tomatoes after all and that the risk in our model is underestimated.

2.2.4 Field decay

Multiplication of viruses does not occur outside a host cell. Therefore only further elimination due to field decay, harvest and storage is considered. Although norovirus and adenovirus are very persistent in the environment, a natural decay process takes place due to environmental factors and time. This natural decay process presumably affects mainly those viruses on the crops' surface, as they are exposed to moisture, salinity, temperature, pH and UV radiation intensity [43]. Since the temperature inside the plants is the same as that on the surface, inner viruses will also suffer temperature decay, although they are more protected against the other factors.

Carratalà et al (2013) found that high temperature was the main cause of adenovirus and MS2 virus inactivation, when compared to UV light [4]. Duizer et al. (2004) found that at 20°C a 3 log reduction in infectivity occurred for calciviruses after 1 week post exposure [9]. More recently, Carratalà et al. (2013) found that MS2, a surrogate for norovirus, on lettuce is inactivated by 2

$\log_{10}$ after 14.67 hours at 30°C under sunlight stimulation [4]. A 3-log reduction was not achieved in their experiments. Dawson et al. (2005) investigated the survival of MS2 as well, and found that after one week at 22°C on lettuce the reduction was 1 $\log_{10}$, while on tomatoes it was minimal [7].

Adenovirus at 30°C is inactivated up to 4 $\log_{10}$ after 8h hours both in the dark and under sunlight simulation [4]. The temperature decay process can be described with equation 2

$$C_{t=}C_0 e^{-kt} \qquad \text{Equation 2}$$

Where C_0 is the initial concentration of virus on the crop's surface or inner part, t is the time, k is the decay constant and C_t is the concentration of virus after time t.

The decay constant for enteric viruses in general was earlier estimated between 0.65 and 0.73 day^{-1} [45, 43]. Petterson et al. (2001a and b) re-considered this estimate via a kinetic study of lettuce during their screening-level risk assessment of wastewater-irrigated salad [28, 29]. They found that 1.07 $days^{-1}$ agreed better with their experimental data. As the exact elapse time in the field in this case is unknown, the model did not consider the decay constant by Pettersson et al. (2001b), but uses the information on temperature decay found by Carratalà et al (2013) [29, 4].

2.2.5 Harvest

Hands of people that touch the produce can contaminate and introduce viruses to the products. These people can be, for instance, harvesters, food handlers, and customers. In this case study, field workers do not wear gloves, and can transmit contaminated water, for instance after doing maintenance tasks of the irrigation system, to the surface of edible parts of the crop during harvesting. In a study on worker's hands in a green pepper bell production chain, 0 of 36 workers had contaminated hands before harvest, whereas five hands (14%) were contaminated after three hours of work [20]. Norovirus was also detected on 9 of 20 green bell peppers coming from the field. The absence of noroviruses from pickers' hands before labor-related activities can be explained by the rinsing of workers' hands and tools with copper sulfate solutions, which is not a common practice. The authors also visually observed that few workers were using gloves as well as improper gloves handling before and after using restrooms.

These findings are important to notice. However, in the model considered in this thesis, wastewater from secondary effluent is used and there is no evidence of the workers hygiene. Cross-contamination by workers therefore is hard to assess and not included in the model.

2.2.6 Post harvest

Post harvesting processes, ranging from storage and rinsing to cutting, are also possible sources of further elimination. The vegetables that are harvested the night before the market are stored in a concrete house located next to the fields. As described in chapter 2.2.4, temperature will further decrease the amount of viruses on and inside the produce. Although the vegetables are stored in darkness, temperatures in summer still may rise above 20°C in the concrete house. The results from Carratalà et al. (2013) on temperature decay in darkness for adenovirus and MS2, a surrogate for norovirus, are used in the model [4].

The market sells the produce from 7 am to 1:30 pm under an awning. This means that viruses are not directly exposed to UV radiation from sunlight, however temperatures on the market, like in the storage room, can rise to about 30°C. This decay is included in the above-mentioned reduction in viral concentrations found by Carratalà et al. (2013) [4].

2.2.7 Consumer habits

Cooking is clearly a fail-safe method for pathogen elimination from produce [24]. However, lettuce and tomatoes are generally consumed raw and without any or minimal preparation. Consumer habits, like washing or storing the vegetables under certain circumstances, have impact on the virus survival rate [49].

Although no information on the prevalence, frequency or intensity of vegetable washing in most societies is available, the literature is reviewed with regard to the effectiveness of post-harvest handling [12]. Drechsel et al. (2010) acknowledges in chapter 12 the effect of disinfection methods on faecal coliform levels on lettuce [49]. The most common method for disinfecting salad is using running tap water or dipping the salad in a bowl of water. The log unit reductions in viral concentrations established by Drechsel et al. (2010) are between 0.3-2.2 and 1.0-1.4 for running tap water and dipping in a bowl of water, respectively. Depending on the quality of the water used for rinsing, this

process can contribute to the virus contamination by addition and removal of viruses. Spain's tap water has a high quality and therefore will rather remove than add viruses to the produce. Recently, Predmore and Li (2011) investigated the efficiency of norovirus removal from fresh vegetables and fruits with different surfactants and sanitizers [31]. Lettuce samples rinsed with tap water only led to 0.23- log reduction in virus titer. However, tap water did not give virus reduction of more than 1 log in any tested fruit or vegetable. The authors concluded that surfactants other than sodium dodecyl sulfate (SDS) significantly enhanced virus removal from fresh produce and that the combination of a surfactant and chlorine was the most effective sanitizer.

Storage of vegetables in the fridge will prolong the survival of viruses on the crops due to low temperature as discussed above (Chapter 2.2.6). However, consumers might store their vegetables under different conditions, which have a different impact on virus survival.

2.2.8 Consumption of (raw) vegetables

The consumption habits of the population of Catalonia, Spain, will mainly determine the risk the population is running by eating wastewater-irrigated vegetables. To estimate the actual risk as accurately as possible, consumption survey data of the Spanish population is used in the model [52]. The survey was conducted during 2009 and 2010. A random selection of 1500 men and 1500 women all over Spain and in every age category were interviewed about their three day dietetic history. The data were categorized into different vegetable groups. The interviews were equally distributed through the four year seasons [52]. As this risk assessment focuses on tomatoes and lettuce, only the data from these categories are used in the model development. The survey data are sorted by consumers and the general population. The average consumption in gram per person per day is given of different percentiles of the population.

The median consumption of lettuce from the whole population is 32.93 with a standard deviation of 26.48g per person per day. Tomatoes are consumed in a higher amount (65.74 with a standard deviation of 55.57g per person per day).

Table 4 Results on vegetable consumption survey in Spain of lettuce and tomatoes from 2009-2010. Values are given in g person^{-1} day^{-1} [52]

		N	%	Median	SD	Consumption 2.5%	Consumption 25%	Consumption 50%	Consumption 75%	Consumption 95%	Consumption 97.5%	Consumption 99%
Lettuce	Total population			20.72	26.35	0	0	11.67	30	74.17	89.17	120
	Consumer population	1888	62.93	32.93	26.48	3.33	13.33	26.67	43.33	83.33	100	126.67
Tomatoes	Total population			55.6	56.35	0	13.33	41.67	80	163.33	200	246.67
	Consumer population	2537	84.57	65.74	55.57	5	26.67	53.33	86.67	168.33	216.67	250

2.3 Effect assessment

In the effect assessment the dose-response relationships for the investigated pathogens is outlined and possible susceptible populations are described.

2.3.1 Dose-response data

Unlike in chemical effect assessment, in the QMRA effect assessment a single pathogen can cause a physiological response. The physiological response to a given pathogen dose may be described with reference to two potential stages: infection and illness [28]. As we are interested in the risk of getting ill from eating wastewater-irrigated food crops, dose-response relationships with the endpoint illness have been investigated. Several dose-response relationships have been established to describe the physiological response for different pathogens.

Teunis et al. (2008) described a dose response relation to estimate the probability of infection and conditional probability of illness given infection for noroviruses [37]. The developed model is a single hit model with model parameters that describe a beta distribution of a single unit (viron). This is the only quantitative model available for QMRA of norovirus to date. The probability of infection for a given norovirus dose D is calculated as:

$$P_{inf}(D|a,b) = 1 - F_1(a, a+b| - D) \qquad \text{Equation 3}$$

Where parameters a and b characterize infectivity, and F_1 is a confluent hypergeometric function. The dose-response relation for the probability of illness conditional of infection is calculated by equation 4:

$$P_{ill}(D|\eta, r) = 1 - (1 + \eta D) - r \qquad \text{Equation 4}$$

With parameters $\eta = 2.55*10^{-3}$ and r= 0.086 [details in 37]. Frequently, the dose-response model may not be very representative for less advanced communities, such as infants and the elderly. Another point of attention by using this dose response model is that the particular strain of norovirus used (Norwalk virus) is considered highly infectious, where this infectivity remains to be assessed for other variants of norovirus.

Crabtree et al. (1997) used an exponential model based on the data obtained from a human dose-response study [5, 6] to describe the risk of infection for adenovirus. Although the study involved human inhalation of small-particle aerosols of adenovirus 4 with respiratory illnesses as health outcome, the

developed exponential dose response model (Equation 5) of Crabtree et al. (1997) is the only one available for adenovirus [6].

$$P_{inf} = 1 - \mathrm{e}^{-\mathrm{rD}} \qquad \text{Equation 5}$$

With r equal to 0.4172 and D representing the number of organism ingested or inhaled. Soller et al (2010) reported that 50% of adenovirus infections result in illness, and used this figure for estimating the risks of gastroenteritis disease caused by adenovirus [36].

Subsequently, for both dose-response relationships, the annual risk (P_y) is based on equation 6, with P_d as the daily risk of getting ill and t as the amount of exposure events in days per year.

$$P_y = 1 - (1 + P_d)^t \qquad \text{Equation 6}$$

2.3.2 Susceptible population

The pitfall of the above described dose-response relationships is that the curves are based on healthy adult volunteers. However, certain subpopulations might experience a higher vulnerability to lower pathogen concentrations than healthy volunteers. Several researchers have investigated the susceptibility to norovirus and adenovirus.

Norovirus gastroenteritis occurs in all ages and in sporadic and epidemic disease patterns. However, children younger than 5 years old are more susceptible than adults [26]. Mortality is rare and has occurred mainly in people with pre-existing conditions and the elderly. Lindesmith and co-workers (2003) found that about 80% of their study population was secretorpositive for the virus [21]. From this 80% about 45% got infected and 35% were protected. On the other hand, none of the secretornegative volunteers got infected after being challenged with different concentrations of norovirus. The authors also observed that complete resistance to Norwalk virus infection is possible for patients with a genetic factor that may code for the virus-binding site. Lindesmith et al (2003) and Teunis et al. (2008) found that people with blood group O were significantly more susceptible to Norwalk virus infection than other blood types [21, 37]. The dose-response model from Teunis et al. (2008) was based on the fact that the secretor-positive subpopulation is susceptible for norovirus [37].

According to Hierholzer (1992), adenovirus infections are associated with case fatality rates as high as 60% in immunocompromised hosts with pneumonia compared with 15% in immunocompetent patients with pneumonia [14].

Adenovirus type 1, 2, 3, 5, 7, and 41 are the most common adenoviruses found in normal children, but these serotypes account for just 53% of adenovirus infections reported for congenitally immunocompromised children [14]. Gastroenteritis is mainly associated with viral genotypes 40 and 41 in children between 1 and 4 years old. Thus, children are a susceptible population for gastroenteritis caused by adenovirus. In patients with primary immunodeficiencies, lung, liver, and kidney are commonly involved in the course of the disease. Due to intensive chemotherapy, cancer patients have a weak immune system, which make them susceptible for adenovirus infection as well [14].

2.4 Risk characterization

The risk characterization of a QMRA integrates the information from hazard identification, exposure assessment, and effect assessment into a single mathematical model to calculate the risk of an outcome like infection, illness or death. Since exposure and effect assessment will not provide a single value, but a distribution of values, the risk needs to be calculated for all values across this distribution. This is done via the Monte Carlo Analysis and will result in a full range of possible risks, including the minimum, average and worst-case scenarios. Based on these risk estimations risk, public health officials may shape their policies regarding the use of wastewater use in irrigation.

The development of the mathematical model is described in chapter 3 of this thesis. In this chapter, input data for the model as well as the distribution of the values are elucidated.

3. Model development

Exposure and effect values from literature reviewed in the previous chapter is used to develop a QMRA model for assessing the risk corresponding to ingestion of tomatoes and lettuce irrigated with tertiary effluent in Catalonia. In this chapter the model input parameters as well as used formulas are listed and discussed. The method is briefly explained. The second part of this chapter outlines the results obtained with the model. Finally, the results of the sensitivity analysis are presented.

3.1 Input data and formulas

For developing a model to assess the risk of illness due to ingestion of wastewater-irrigated vegetables, scientific data from the literature are combined with relevant formulas. A summary of all input parameters used for the model development for norovirus and adenovirus can be found in Table 6 and 7, respectively.

The data measured by Katayama et al. (2008) in chlorinated secondary effluent from June until September are used as the initial norovirus concentration [19]. This is the only study where summer concentrations of noroviruses are reported. Because both genotypes I and II are human pathogens, the sum of their averages is used. A normal distribution was applied to the log of the data and the sigma of the distribution was chosen so that the minimum (-1.49 $\log_{10}$ RT-PCR units ml^{-1}) and the maximum (-0.25 $\log_{10}$ RT-PCR units ml^{-1}) concentrations reported were covered by the 1^{st} and the 99^{th} percentiles, respectively. For adenovirus, the data from Bofill-Mas et al. (2006) are selected as these measurements were taken in Spain [3]. Although the data are obtained in winter, its use was considered appropriate for this study because adenoviruses do not show seasonal variation [19]. The average of Bofill-Mas' data from assay 1 are used because this assay achieved more reliable data than assay 2 according to the authors themselves [3]. A normal distribution was applied to the log-data with a mean of 0.67 RT-PCR units mL^{-1} and a sigma of 0.67 as suggested by the authors.

As both initial concentrations used in the model are measured from secondary effluent rather than tertiary effluent, the effects of UV and chlorine on virus inactivation were assessed. The initial concentration of norovirus was measured after chlorination, so only a UV reduction was necessary in this case. A point

estimate of 4 $\log_{10}$ reduction is used according to Hijnen et al. (2006), who reported a reduction of this magnitude in animal caliciviruses for the fluences usually applied in UV treatment by wastewater treatment plants (WWTP) [15].

Adenoviruses are very resistant to UV radiation. Hence, a uniform distribution with a maximum of 1 $\log_{10}$ reduction was used, based on the literature review from Hijnen et al. (2006) [15]. However, different strains of one virus species may have different UV sensitivity and micro-organisms may still retain metabolic functions after UV exposure. Gerba et al. (2002) demonstrated that adenovirus type 2 is more resistant than adenovirus types 40 and 41 [11]. Type 2 causes mainly respiratory illnesses, but we are interested in gastrointestinal illnesses. Thus, the data from Hijnen et al. (2006) are more relevant than data from Gerba et al. (2002) [15, 11]. Despite that, a uniform distribution from 0-1 $\log_{10}$ is used in the model to account for this uncertainty.

Chlorination reduction with a triangle distribution from 3 to 5 $\log_{10}$ is applied to the concentration of adenovirus [39]. The treatment plant in Catalonia uses a free chlorine concentration of about 1 to 3 mg/L for 15 minutes, meaning C_t values are reached between 15 and 45 mg min L^{-1} (see Equation1). According to Thurston-Enriquez et al. (2003a), this would correspond to a $\log_{10}$ reduction of about 3 to 5 for treated groundwater at pH 8 and 15°C [39]. Although the plant in Catalonia operates at an average pH of 7.6 and temperatures may reach 30°C in summer, these are the most relevant data found in the literature as they are comparable to the conditions in Spain.

To calculate the resulting concentration of the log-reductions, formula 7 is used.

$$C = log\, C_0 - log(reduction) \qquad \text{Equation 7}$$

With C_0 being the concentration in secondary effluent or chlorinated water.

This exposure model considers overhead irrigation for lettuce and drip irrigation for tomatoes. Lettuces receive a large amount of water on the surface due to sprinkler irrigation. Furthermore, viruses in the water deposited on the soil around the lettuces could be internalized through the roots. The tomato plants, on the other hand, will receive most irrigation water at the roots; therefore, the crop surface is less contaminated. However, aerosols from the sprinklers located nearby will also contaminate the surface of the tomatoes.

The literature review demonstrates that adenovirus is unlikely to internalize via the roots and reach the edible parts of the crops (fruits and leaves) [16].

Therefore, the internalization in the adenovirus model is not considered for both tomatoes and lettuce. On the contrary, studies on norovirus internalization led to different results. Although most experiments were performed under greenhouse conditions, with high initial concentrations, and different types of lettuce, an internalization ratio of 0.24 was used in the model. This ratio is derived from the DiCaprio study (2012), who found 6.9 x 10^5 RNA copies g^{-1} of human norovirus in the leaf tissue of lettuce grown hydroponically at one day post inoculation of the growth media with 2.9 x 10^6 RNA copies mL^{-1} [8].

Since RNA products do not correspond with the amount of infective viruses, an infectivity ratio of 0.0205 was used to transform the RT-PCR units of norovirus into infective plaque forming units (pfu) g^{-1}. This was based on murine norovirus concentrations in lettuce leaves grown hydroponically [44].

For the amount of virus-containing water clinging to the surface, the experimental data of Shuval et al. (1997) are used [35]. A factor of 0.108 mL g^{-1} is used for lettuce as a normal probability density function with a sigma of 0.019 mL g^{-1} [12, 2]. For tomatoes, the clinging data on cucumber (0.0036 mL g^{-1}) found by Shuval et al. (1997) are used, although the amount is higher than it would be expected to deposit via aerosols formed in the lettuce field [35]. The smooth structure of tomatoes is more similar to the surface of cucumber than to the lettuce.

Viruses in the crops and on the surface suffer time-dependent inactivation. The degree of inactivation, conditioned by sunlight and high temperatures, depends on the virus type. Internalized viruses do not undergo sunlight inactivation, but are affected by high temperatures. It is assumed that the temperature in the crop is the same as that in the surrounding air. Viruses are subjected to inactivation in the field, but also during transport and storage. In the literature review the decay constant from Petterson et al. (2001a and b) is given for the decay calculation [28, 29]. However, this constant is based on enteroviruses. On the contrary, Carratalà et al (2013) reported inactivation values with regards to temperature and sunlight for norovirus surrogates and adenovirus [4]. Table 5 shows the probable duration of the phases after irrigation, and the time, sunlight, temperature virus log inactivation in each phase, based on Carratalà et al (2013) [4].

Table 5 Duration of the phases after irrigation, and the assumed time, sunlight, and temperature virus log inactivation in each phase, based on Carratalà et al. (2013) [4].

Period		**Time (h)**	**Sunlight (h)**	**Temperature (ºC)**	**Log Inactivation**	
					NoV	AdV
Irrigation-harvest		12-36	6-18 (affects only surface viruses)	20-30	Light: 1-2 Dark: 0-1	1-4
Storage	Chamber	24	No	4	0	0
	Concrete house	12	No	20-30	0-1	1-4
	No storage	0	-	-	0	0
Market		1-7	No	20-30	1	1-4
Consumer's house		12-24	No	4	0	0

Surface viruses will be under the influence of sunlight between 6 and 18 hours. Surface noroviruses will suffer a decay of 1 to 2 logs. In the dark, and for internalized norovirus, the inactivation is between 0 and 1 logs. For Adenoviruses, the inactivation is very similar in the dark or in the sunlight, and they suffer a 1 to 4 logs inactivation during the 12 to 36 hours period in the field. After harvest, crops stored for 24h at 4 ºC do not present further viral inactivation, but for those in the concrete house, the inactivation pattern is the same as in the field without the effects of sunlight. The same happens in the market, since vegetables are sold under an awning. In the consumer's household, vegetables are kept in the fridge, so no further inactivation occurs.

The total elapsed time is between 19 hours and four days, but elapsed time in which inactivation happens (so, excluding storage times at 4ºC) is between 13 hours and 2.3 days. Although Carratalá et al (2013) did not continue measurements after 24 hours, most inactivation showed a monophasic trend [4]. For example, adenovirus inactivation in the dark at 30ºC was stable at 3 logs after 2.54 hours. Therefore, we assumed an inactivation between 1 and 2 logs for Norovirus on surface, between 0 and 1 for internalized norovirus, and 1 to 4 logs for adenovirus for the whole period, from last irrigation to consumption.

Post-harvest inactivation via washing with tap water was estimated by Predmore and Li (2011) [31]. Their findings are implemented in a Pert-distribution with 0.1 $\log_{10}$ as minimum, 2 $\log_{10}$ as maximum and 1 $\log_{10}$ as most likely for describing the reduction of post-harvest handling. Although the study

was performed for lettuce and a surrogate for norovirus, the results are also used to model adenovirus with lettuce and tomatoes. Furthermore, we assumed that tomatoes are not peeled, and that viruses are homogeneously distributed on lettuce surface and insides, so removing outer parts will not lead to a change of concentration on the eaten parts.

The vegetables consumption habits of the Spanish population were surveyed by the Spanish agency for food safety and nutrition (AESAN). Their consumption survey data are used in this model [52]. Table 4 (Chapter 2.2.8) shows the results of that interview on three random days during the survey period. The categorized data of lettuce and tomatoes are used with a cumulative distribution of the evaluated percentiles with a maximum of 126 and 250 g person^{-1} day^{-1}, for lettuce and tomato, respectively.

The dose-response models for norovirus disease and adenovirus infection are described by equation 4 and 5, respectively. The parameters for the norovirus model are derived from Teunis et al (2008) [37]. The exponential model parameter r for adenovirus is determined by Crabtree et al. (1997) [6]. With these dose-response models the daily risk of getting ill/infected was calculated. According to Soller et al (2010) 50% of infections of adenovirus result in illness, therefore the daily risk of adenovirus infection was multiplied by 0.5 to obtain the daily risk of getting ill [36].

Since the survey data on consumption used in this model are averages from the whole year, daily variability in consumption is included. The irrigation of the produce mostly occurs in the summer months (May till November). Hence, the parameter t in Equation 6 was substituted by an exposure event of 214 days. Subsequently, the annual risk of getting ill with regards to different scenarios was calculated.

Table 6 Model input parameters and distribution for the norovirus model with lettuce/tomatoes.

Notation and definition (units)	Distribution type (values)	Source
C= norovirus concentration of secondary effluent after chlorination	Normal of log data (-0.8729; 0.55)- truncated max at 1	[19]
R_{UV}= viral $\log_{10}$ reduction from UV light treatment	Point estimate (4)	[15]
Internalization= internalization ratio to edible parts of the plant	0.2379	[8]
Infectivity= infectivity ratio	0.0205	[44]
$I_{temperature}$= viral $\log_{10}$ reduction due to temperature inside the plant	Uniform (0; 1)	[4]
N_{cling}=amount clinging to outer part of the crop (ml g^{-1})	Normal (0.108; 0.019)- truncated min at 0 and max at 0.17 (for lettuce) Normal (0.0036; 0.0015)- truncated min at 0 and max at 0.01 (for tomatoes)	[12] and [2] [35]
T= time between irrigation and consumption (hours)	6-72	Assumption
$R_{temperature}$ = viral $\log_{10}$ reduction due to temperature and sunlight in field and during storage, transport and sale	Uniform (1; 2)	[4]
$R_{washing}$= viral $\log_{10}$ reduction from post-harvest preparation	Pert (0.1; 1; 2)	[31]
Consumption = average daily consumption of lettuce/tomatoes per person (g $person^{-1}$ day^{-1})	Cumulative (see Chapter 2.2.8)	[52]
parameters for Beta-Poisson model	ŋ = 2.55*10-3 and r= 0.086	[37]
t_{year}= days of annual exposure	214	Assumption

•

Table 7 Model input parameters and distribution for the adenovirus model with lettuce/tomatoes.

Notation and definition (units)	Distribution type (values)	Source
C= adenovirus concentration of secondary effluent	Normal of log data (0.67, 0.67) - truncated max at 3	[3]
R_{UV}= viral $\log_{10}$ reduction from UV light treatment	Uniform (0,1) ~0.5 (mean)	[15]
$R_{Chlorination}$= viral $\log_{10}$ reduction from chlorination treatment	Triangular of log data (3, 4, 5)	[39]
Internalization= internalization ratio to edible parts of the plant	0	[1]
N_{cling}=amount clinging to outer part of the crop (ml g^{-1})	Normal (0.108, 0.019)- truncated min at 0 and max at 0.17 (for lettuce) Normal (0.0036, 0.0015)- truncated min at 0 and max at 0.01 (for tomatoes)	[12] and [2] [35]
T= time between irrigation and consumption (hours)	6-72	Assumption
$R_{temperature}$ = viral $\log_{10}$ reduction due to temperature and sunlight in field and during storage, transport and sale	Uniform (1; 4)	[4]
$R_{washing}$= viral $\log_{10}$ reduction from post-harvest preparation	Pert (0.1; 1; 2)	[31]
Consumption = average daily consumption of lettuce/tomatoes per person (g $person^{-1}$ day^{-1})	Cumulative (see Chapter 2.2.8)	[52]
Parameters for exponential model	r = 0.4172	[6]
Infection to illness ratio	0.5	[36]
t_{year}= days of annual exposure	214	Assumption

3.2 Method

For modeling various scenario outcomes, the Monte Carlo simulation method is used. This method builds up successive scenarios using input values that are randomly selected from the input probability distributions, utilizing the computer software @RISK Version 6 for Excel Launcher. For each run, the software draws one random variable from the distribution for each of the model input variables and computes a single result. A large number of repeated computations produce a complete distribution of the modeled results. A total of 10000 Monte Carlo iterations are done per simulation to guarantee convergence and to make up a representative sample of the near infinite number of combinations of possible input variables. The Monte Carlo simulation method produces a complete distribution of output variables, of which the mean, the 95% lower confidence limit (LCL) and the 95% upper confidence limit (UCL) are reported.

A Sensitivity analysis is used to identify model parameters (inputs) providing the greatest contribution to the risk of virus illness of raw vegetables. The Spearman rank correlation coefficient is used to establish the correlation between the model output and each input. The input data with a higher influence on the risk are identified.

4. Model output

The model estimates a concentration of norovirus and adenovirus in the irrigation water of 1.34 x 10^{-5} pdu/mL and 1.48 x 10^{-4} pdu/mL, respectively. Results for average concentration and risk of norovirus and adenovirus at each step along the source-to-fork timeline for both lettuce and tomatoes are shown in table 8 and 9, and in figure 2.

Table 8 Concentrations and risks of norovirus at certain steps in the source-to-fork approach for lettuce and tomatoes. Arithmetic average values and 90% confidence interval of the distributions in brackets are given. For the crops, surface, internalized and total virus concentrations are indicated. i.v.= infective virus counts; pppd = per person per day; pppy = per person per year

	Lettuce			**Tomatoes**		
	Surface	**Internalized**	**Total**	**Surface**	**Internalized**	**Total**
Concentration in tertiary effluent (RT-PCR ml^{-1})	-	-	2.94×10^{-5} (1.67×10^{-6}–1.07×10^{-4})	-	-	2.94×10^{-5} (1.67×10^{-6}–1.07×10^{-4})
Concentration after irrigation (i.v. g^{-1})	6.49×10^{-8} (3.58×10^{-9}–2.38×10^{-7})	1.43×10^{-7} (8.14×10^{-9}–5.23×10^{-7})	2.08×10^{-7} (1.17×10^{-8}-7.61×10^{-7})	2.18×10^{-9} (8.97×10^{-11}-8.38×10^{-9})	1.43×10^{-7} (8.13×10^{-9}–5.22×10^{-7})	1.45×10^{-7} (8.23×10^{-9}-5.31×10^{-7})
Concentration after sunlight and/or temperature inactivation (i.v. g^{-1})	2.50×10^{-9} (8.93×10^{-11}–9.82×10^{-9})	5.69×10^{-8} (1.92×10^{-9}–2.19×10^{-7})	5.94×10^{-8} (2.01×10^{-9}-2.29×10^{-7})	4.21×10^{-9} (1.06×10^{-10}-1.66×10^{-8})	5.72×10^{-8} (1.93×10^{-9}–2.24×10^{-7})	6.14×10^{-8} (2.04×10^{-9}-2.41×10^{-7})
Concentration after washing (i.v. g^{-1})	3.32×10^{-10} (5.83×10^{-12}–1.39×10^{-9})	5.69×10^{-8} (1.92×10^{-9}–2.19×10^{-7})	5.72×10^{-8} (1.93×10^{-9}-2.20×10^{-7})	5.46×10^{-10} (7.32×10^{-12}-2.24×10^{-9})	5.72×10^{-8} (1.93×10^{-9}–2.24×10^{-7})	5.77×10^{-8} (1.94×10^{-9}-2.26×10^{-7})
Dose (i.v. person^{-1} day^{-1})	-	-	1.26×10^{-6} (1.98×10^{-20}–5.42×10^{-6})	-	-	3.36×10^{-6} (1.28×10^{-8}-1.35×10^{-5})
P_d Illness (pppd)	-	-	2.76×10^{-10} (0-1.19×10^{-9})	-	-	7.36×10^{-10} (2.80×10^{-12}-2.95×10^{-9})
P_y Illness (pppy)	-	-	5.91×10^{-8} (0-2.55×10^{-7})	-	-	1.58×10^{-9} (6.0×10^{-10}-6.32×10^{-7})

Table 9 Concentrations and risks of adenovirus at certain steps in the source-to-fork approach for lettuce and tomatoes. Arithmetic average values and 90% confidence interval of the distributions are given. For the crops total virus concentrations are indicated. pdu = polymerase chain reaction detectable units ; pppd = per person per day; pppy = per person per year

	Lettuce	**Tomatoes**
Concentration in tertiary effluent (pdu ml^{-1})	8.75×10^{-4} (6.23×10^{-6}-3.33×10^{-3})	9.36×10^{-4} (6.18×10^{-6}-3.75×10^{-3})
Concentration after irrigation (pdu g^{-1})	9.62×10^{-5} (6.66×10^{-7}-3.52×10^{-4})	3.34×10^{-6} (1.73×10^{-8}-1.24×10^{-5})
Concentration after sunlight and temperature inactivation (pdu g^{-1})	1.36×10^{-6} (5.09×10^{-10}-4.56×10^{-6})	5.42×10^{-8} (1.50×10^{-11}-1.54×10^{-7})
Concentration after washing (pdu g^{-1})	2.04×10^{-7} (3.91×10^{-11}-5.54×10^{-7})	7.74×10^{-9} (1.28×10^{-12}-1.72×10^{-8})
Dose (pdu $person^{-1}$ day^{-1})	5.44×10^{-6} (1.30×10^{-21}-8.9×10^{-6})	4.86×10^{-7} (9.49×10^{-12}-8.60×10^{-7})
P_d Illness (pppd)	1.13×10^{-6} (0-1.87×10^{-6})	1.01×10^{-7} (1.98×10^{-12}-1.79×10^{-7})
P_y Illness (pppy)	2.24×10^{-4} (0-4.0×10^{-4})	2.16×10^{-5} (4.24×10^{-10}-3.84×10^{-5})

The annual risk outcome of norovirus and adenovirus concerning consumption of tomatoes and lettuce irrigated with tertiary effluent is shown in table 8 and 9. The results show that the average risk of getting ill due to consumption of lettuce (5.91×10^{-8} and 2.24×10^{-4}) is higher than for tomatoes (1.58×10^{-9} and 2.16×10^{-5}). In the analyzed scenarios, the risk associated with adenovirus (2.55×10^{-7} and 1.58×10^{-9}) is higher than for norovirus (2.24×10^{-4} and 2.16×10^{-5}).

The highest average risk of getting ill is due to consumption of adenovirus contaminated lettuce (2.24×10^{-4} pppy). The illness risk after ingestion of norovirus contaminated tomatoes is the lowest one (1.58×10^{-9} pppy). The differences in risk of about 5 log units between these two scenarios are mainly attributed to the different irrigation systems used. Lettuce receives more irrigation water on the surface than tomatoes do. Therefore, the risk is higher for adenovirus contaminated lettuce than for norovirus contaminated tomatoes, although the population consumes more tomatoes than lettuces (Table 4).

A large proportion Norovirus is internalized which is then hardly removed during post-harvest treatment. This results in a higher dose of norovirus than adenovirus. The higher concentration of noroviruses in tertiary effluent also contributes to this fact. However, due to the difference in dose-response, the estimated risk for adenovirus is higher than for norovirus.

According to the WHO, a yearly risk of 10^{-6} DALY loss pppy due to viral, bacterial and protozoan disease resulting from consumption of wastewater-irrigated crops is acceptable [WHO 2010]. The equivalent tolerable disease risk pppy equivalent for rotavirus in industrialized countries is 7.1×10^{-5}. The average estimated risks in the analyzed scenarios are below this limit. However, the upper confidence limit and the average of the risk of adenovirus in lettuce is above it.

The total annual risk outcome of norovirus and adenovirus concerning consumption of tomatoes and lettuce together irrigated with tertiary effluent is calculated with equation 8:

$$P_{y\,total} = 1 - [(1 - P_{d\,lettuce})^t \, x \, (1 - P_{d\,tomato})^t] \qquad \text{Equation 8}$$

The average total annual risk of gastrointestinal disease via norovirus due to ingestion of contaminated tomatoes and lettuces is 2.17×10^{-7} (5.99×10^{-10}-8.86×10^{-7} pppy). For gastrointestinal disease via adenovirus due to ingestion of

contaminated tomatoes and lettuce, the average total annual risk is 2.63 x 10^{-4} (4.24 x 10^{-10}-4.38 x 10^{-4} pppy).

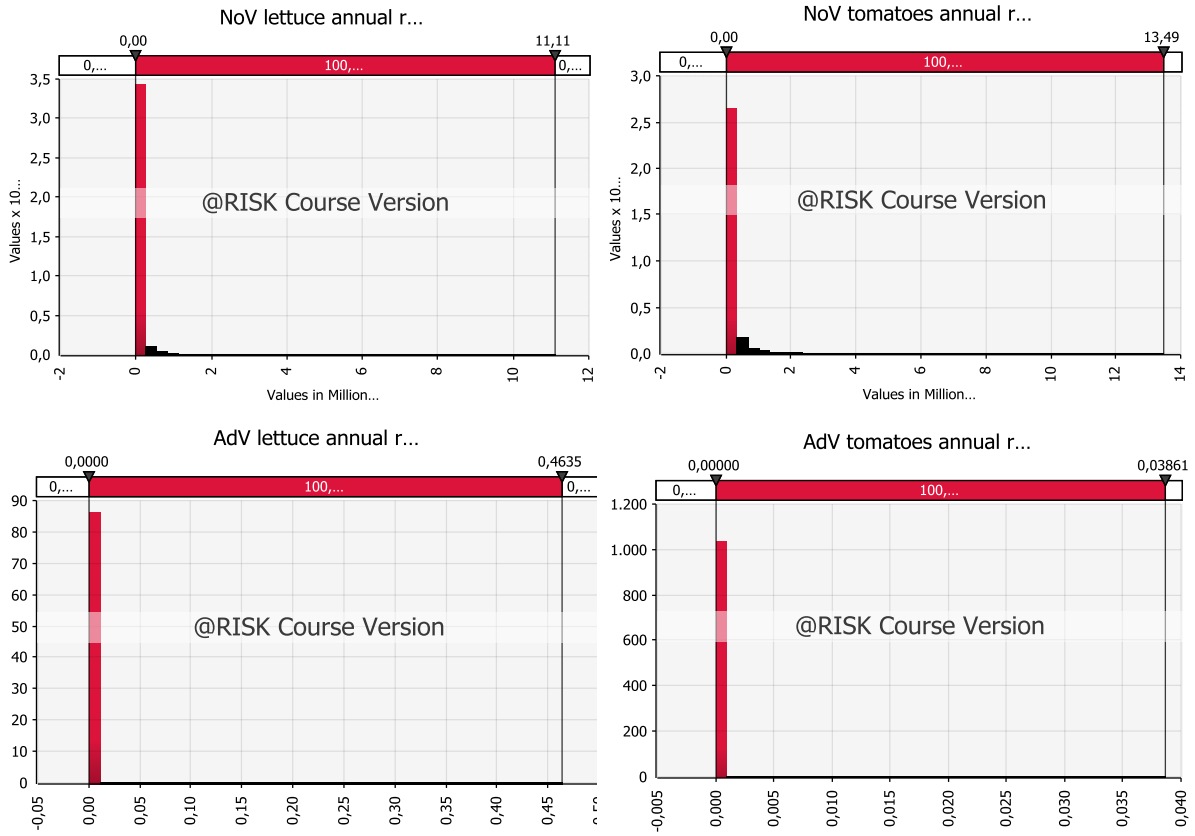

Figure 3 Annual probability density risks of ingestion of tertiary effluent irrigated tomatoes and lettuces. The graphs are derived from a Monte Carlo simulation with 10000 iterations. On the Y-axis the probability densities of the corresponding risks (X-axis) are plotted.

4.1 Sensitivity analysis

A Sensitivity analysis was used to identify model parameters (inputs) providing the greatest contribution to the risk of virus illness of raw vegetables. As shown in table 10, the risk of norovirus disease due to norovirus infection is mostly influenced by the crops consumption and the virus concentration in the secondary effluent, followed by the effects of temperature inside the crops, for both lettuces and tomatoes. However, the effect of consumption is higher on lettuces than on tomatoes.

The risk of adenovirus is highly affected by the consumption of lettuce, followed by the surface temperature inactivation, and the concentration in the secondary effluent. For adenovirus on tomatoes, the temperature reduction is the main factor, followed by the concentration in secondary effluent and the

consumption. A negative correlation between the annual risks and reductions during the source-to-fork chain is more evident for adenovirus than for norovirus. Hamilton et al. (2006) also found consumption to be the most significant determinant of the annual risk [12].

Table 10 Sensitivity analysis of the risk simulations. Correlation of inputs with annual risk output according to Spearman Rank. Only relevant correlations are reported.

		Norovirus		**Adenovirus**
Lettuce	**Consumption**	0.83	**Consumption**	0.71
	Conc. in secondary effluent	0.38	**Temperature reduction on surface**	-0.36
	Internal temperature reduction	-0.20	**Conc. in secondary effluent**	0.28
			Chlorination	-0.16
			Post-harvest treatment	-0.15
			UV	-0.12
Tomatoes	**Consumption**	0.66	**Temperature reduction on surface**	-0.59
	Conc. in secondary effluent	0.62	**Conc. in secondary effluent**	0.43
	Internal temperature reduction	-0.32	**Consumption**	0.42
			Chlorination	-0.27
			Post-harvest treatment	-0.23
			UV	-0.20
			Clinging amount	0.12

5. Discussion

This thesis is an assessment of the health risks associated with the consumption of raw agricultural products irrigated with reclaimed wastewater using a probabilistic QMRA model. Concentrations of adenoviruses and noroviruses in source water (secondary effluent), and their transmission and decay in every step of the source-to-fork chain were assessed using published scientific data. This information was combined with population consumption survey data to obtain an exposure dose, subsequently used to estimate the population risk. All assessed risks except for adenovirus on lettuce was below the maximum tolerable risk defined by Drechsler et al. (2010) for virus, protozoan and bacterial diseases originated from consumption of wastewater-irrigated crops [49]. However, system failure at the WWTP level might result in lower virus inactivation and higher health risks.

Most input values chosen for the model are conservative and may not represent the true value. The calculated risks are therefore likely to be overestimated. Nevertheless, a few assumptions, however, may underestimate the illness risk (e.g. internalization of adenovirus). The sensitivity analysis shows that certain input parameters like consumption and concentration in secondary effluent have a higher influence on the model outcome. To improve the model, efforts have to be directed to the inputs that have higher impact on the output. These are consumption characteristics of the population, concentration of viruses in secondary effluent and temperature-dependent inactivation of viruses. Ideally, the virus concentration should be measured in the tertiary effluent of the investigated WWTP and the consumption data should come from the specific population of interest.

The average and standard deviation of norovirus in secondary chlorinated effluent used in the present study correspond to measurements done in Japan from June to September (Katayama). This model includes months from May to November. This may lead to an overestimation on norovirus concentrations in water in the months of May, October and November.

Since norovirus cannot yet be cultured in any known cell line [34], to date, most of the understandings of the stability and persistence of human norovirus comes from studies of surrogate viruses, like MS2, FVC, MNV, and other CV. Although these animal caliciviruses share various degrees of genetic relatedness

with noroviruses, they differ from human noroviruses in clinical manifestations, human host receptors, pathogenesis, and immunity [8]. Therefore, assumptions made on transmission and inactivation of noroviruses based on these surrogates may significantly differ from the true values.

It has been suggested that viruses can reconstruct themselves via dark repair, photo-reactivation mechanisms or repair enzymes of their host cells after being damaged by e.g. UV light or chlorination [15, 38]. These repair mechanisms could lead to higher concentrations of infective viruses in the tertiary effluent and the crops. Another point of consideration is that noroviruses are able to recombine in the environment which might lead to new pathogens and, therefore, unpredicted risks.

A failure in the tertiary treatment system, would lead to a higher concentration of pathogens in the effluent. The plant in Spain has introduced techniques to prevent too high concentrations in the tertiary effluent due to failures, allowing the high concentrations for a limited period of time. The risk during the failure of the system has not been assessed in this risk assessment. Nevertheless, it is likely that the daily risks will increase for the time the failure occurs.

Irrigation of vegetables as considered in this model, represents a high reuse activity and may not be indicative of risks associated with other uses. The clinging ratio from water to crops surface used in the model has been derived from a study in which lettuces were completely immersed in water [35]. This might lead to higher volumes of water trapped on the surface than through overhead spray irrigation. However, that is the only published study were the water captured by lettuces surface has been assessed, and it has previously been used by other authors with the same assumptions [12, 2].

Tomatoes can receive aerosols from the nearby located sprinklers and splashing of soil water (rain and workers), and also contamination from insects or workers' hands [1]. The load of viruses that the tomatoes receive on the surface through this path has been represented by a ratio derived from immersion of cucumbers in water. This might pose an overestimation of the viruses on tomatoes' surface and, therefore, on the health risks.

The estimation of viruses attachment based on the volume of water clinging to the crop following irrigation, does not consider virus adsorption behavior and immediate inactivation. Also the tendency of some enteric viruses to clump

together or around particulate matter in the soil is not considered in the model [12].

Internalization data used in the model are derived from studies done in hydroponic solutions. In soil, however, internalization of virus has barely ever shown to happen, probably due to attachment of viruses to soil particles, which decreases the probability of roots absorption [1]. On the other hand, virus internalized through stomata and surface wounds are not taken into account. Urbanucci et al. (2009) argued that the barrier against internalization is reduced when the root system is damaged [41].

In this model, only the load of viruses on crops' surface and in soil due to the last irrigation event prior to harvesting are taken into account. However, irrigation happens every two days and, although inactivation of viruses takes place, a portion of them might remain infective. This can lead to an underestimation of the risks.

The dose-response model used for adenovirus has been derived from inhalation of adenovirus type 4 causing respiratory illness [6]. Adenovirus type 40 and 41 causing gastroenteritis disease may show a different dose-response relationship. However no specific dose-response model has been derived for gastrointestinal adenovirus. The dose-response model for norovirus from Teunis et al. (2008) may not be very representative for less advanced communities, such as infants or the elderly [37]. Another point of attention by using this dose response model is that the particular strain of norovirus used (Norwalk virus) is considered highly infectious, where this infectivity remains to be assessed for other variants of norovirus.

In addition, the higher susceptibility of immunocompromised people, children, elderly, and pregnant women, to infections is not taken into account and, hence, the risk for this population fraction has been underestimated. For obvious reasons, dose-response data for these susceptible populations does not exist.

Only two parameters varied between vegetable types in the model, which were the amount of water clinging to the surface, and the population consumption. However, other parameters are presumed to vary, between vegetable types, which are not included in the model. These are, for instance, root intake of viruses or virus survival or decay on surface or in the plant's tissues [12].

No information has been found in the literature about the frequency and intensity of salad-vegetables washing habits of the population, neither on the use of disinfectants. Specific population information would be of great value for the improvement of the model.

Cross-contamination during harvesting has been considered negligible. This is because reclaimed is presumed to be more contaminated than workers hands. However, the relatively low amount of water clinging to the surface could lead to comparative concentrations of pathogens on workers' hands, especially if they are disease carriers. This pathway should, therefore be studied more thoroughly.

Results are not possible to compare because of differences in viruses used, decay rates, population consumption data, risk expression (infection, disease, DALYs) or other assumptions [12]. Expression of the risks in DALYs would be a better way of expressing risk outputs, because it considers different disease outputs and weights the severity of each disease. This allows for comparison between several health conditions [49]. DALYs have not been calculated in the present study because of the lack of published information on the years of life lost due to adenovirus illness. The probability of illness per person per year has been, instead, compared to the rotavirus probability of illness per person per year needed to achieve the acceptable DALYs of 10^{-6} [49]. Rotavirus is an enteric virus that causes diarrhea. Differences in symptoms severity between rotavirus and norovirus or adenovirus may lead to different DALY output.

6. Conclusions and Recommandations

The estimated risk of norovirus and adenovirus after ingestion of crops irrigated with tertiary effluent is low according to the WHO recommendations. The highest risk was obtained for adenovirus on lettuce, followed by adenovirus on tomatoes, norovirus on lettuce and norovirus on tomatoes. All reported average risks are within the boundaries of the WHO recommended annual risk of 10^{-6} DALYS for consumption of wastewater irrigated raw eaten produce, except for the risk of adenovirus contaminated lettuces. The latter is likely to be overestimated, due to the conservative approach used for modeling the amount of virus containing water that clings to the surface after overhead spray irrigation. Although this parameter was only sensitive for adenovirus contaminated tomatoes with regards to the performed sensitivity analysis, the conservative approach has influence on the risk outcome.

Despite the large amount of available literature data for developing QMRA models to assess the disease risks of ingesting crops irrigated with reclaimed water, knowledge gaps, like initial concentration in the effluent of the WWTP or amount clinging to the surface of the produce, exist that have to be specifically managed. Ways to address these data gaps (e.g. the use of surrogate viruses, or the use of the best data available) may lead to under- or overestimation of the disease risk. Although data from virus surrogates and assumptions are used in the model, the developed model can be used as a framework for developing a more accurate QMRA for the situation in Catalonia, once the missing data becomes available.

The author recommends the use of specific microbial data measured in the tertiary effluent of the assessed WWTP as irrigation source for the model- if available. It is also suggested to run more diverse scenarios to evaluate the entire risk of getting ill associated with ingestion of raw vegetables from the investigated farms. Treatment failure scenarios, as well as additional contamination sources should be considered for improving the model.

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