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Impact of Climate Change on Food- and Water-borne Infectious Diseases in Europe

Decision-making tool

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Summary

Food- and water-borne infectious disease incidences may be impacted by climate changes but to which extent is yet unclear. To assess relative risks of climate change-associated food- and water-borne diseases for EU member states, a decision-making tool based on mathematical models is developed. To this purpose, a conceptual framework was developed exhibiting the diverse and complex transmission routes for food- and water-borne diseases under the influence of climate factors. Food- and water-borne diseases are caused by pathogens that are transported through food, water and soil. The characteristics, fate and behaviour of these pathogens are described for selected pathogens. This selection for the tool includes *Salmonella*, *Campylobacter*, *Vibrio*, *Cryptosporidium* and norovirus. Humans and animals may serve as reservoirs for food- and water-borne pathogens that can be transmitted through the environment. The major climate factors that may affect the die-off, survival, inactivation and/or growth of pathogens are temperature and sunlight. Rainfall affects transport of water-borne pathogens and run-off of pathogens and organic material from surfaces into water. These environmental, climate and pathogen factors acted as the building blocks for the eventual decision-making tool following a quantitative microbial risk assessment approach. In total, thirteen QMRA combinations are available in the tool, each existing of a set of consecutively linked modules selected from the 22 modules available. These thirteen QMRA combinations can be fed with location-specific current climate conditions, projected climate conditions and specific data depending on the selected modules, to estimate the direction (increase or decrease) and size of the relative infection risks for the selected pathogens caused by climate change. This tool can assist local public health authorities to assess the impact of climate change on the selected food- and water-borne pathogens in their region.

1 Introduction

Concerted action is needed to address public health issues raised by climate change (Semenza and Menne, 2009). Climate change is likely to have a wide-ranging and adverse impact on human health which may be direct or indirect. Direct effects include extreme weather conditions, such as floods, and sea level rise e.g. leading to an increased risk of drowning and temperature related effects, such as heat waves and smog. An increased frequency or severity of heat waves would cause an increase in mortality and illness (McMichael et al., 2001). Indirect effects may include the introduction of invasive species releasing aeroallergens and therefore allergies. Another indirect effect would be spoilage due to increased air temperature leading to food-borne disease. Water-borne disease incidences may also be influenced by climate change. For example, increased temperature can influence growth and die-off or inactivation of water-borne pathogens.

Consequences of climate changes may be unidirectional or bidirectional. An example of the former is an increase or decrease in disease incidences due to climate change. An example of a bidirectional effect is increased air temperature causing heat waves in summer, leading to higher disease burden due to the harvest effect (people die sooner than they would have without this factor), whereas warmer winters in temperate climates may lead to a decreased loss of lives. Similarly, pathogens that cause food- and water-borne diseases may be affected quite diversely by climate changes, with either increasing or decreasing adverse health outcomes.

Associations between global warming and public health outcomes are assumed to be causal. However, accurate scientific predictions of the true human health outcomes of global climate change are scarce and often significantly confounded by diverse effect modifiers. Adaptation strategies are not always straightforward due to the complexity of (micro)biological processes that can be affected by climate change. Therefore, the use of quantitative microbial risk assessment (QMRA) may guide adaptation strategies and aid their prioritization by estimating relative public health risks (Schijven and de Roda Husman, 2005). To this end, QMRA requires extensive knowledge of the dynamics of climate change and infectious disease but also of quantitative modeling. To aid public health officers in European member states, a decision-making tool based on mathematical models will be developed in the framework of ECDC project *Impact of Climate Change on Food- and Water-borne Diseases in Europe* Lot 2 to assess relative risks of climate change-associated food- and water-borne disease. Insights from these analyses could then guide adaptation strategies and protect public health from impending threats related to climate change.

1.1 Outline

This report describes the building blocks that are needed for the design of the decision-making tool for relative assessment of the impact of climate change on food- and water-borne disease in the different European member states. In Lot 1 of the ECDC project ‘Impact of climate change on food- and water-borne diseases in Europe’, a number of pathogens was selected for which the impact of climate change will be assessed. Chapter 2 gives descriptions of the occurrence and characteristics of these pathogens. Chapter 3 gives an overview of the sources for food- and water-borne pathogens that may be subject to climate change. Chapter 4 presents a conceptual model which identifies the major pathways of food- and water-borne pathogens in the environment and the climate changes that may affect pathogen fate and behaviour. In chapter 5, the basics of Quantitative Microbial Risk Assessment (QMRA) are explained. Furthermore, a general description of, and approach to the development of modules that

describe a change in fate and behaviour of the selected pathogens as a consequence of climate change are given. Chapter 6 describes the plan for the development, testing and delivery of the modules.

2 Selected pathogens

In Lot 1 of ECDC project *Impact of Climate Change on Food- and Water-borne Diseases in Europe* pathogens were selected based on factors in one of three different categories of criteria: 1) extent of the health risk; 2) food- and water-borne transmission; and 3) data needs for QMRA (Table 1). For instance, with regard to disease outcome, each of the selected pathogens may cause gastroenteritis making up for the largest part of causative agents for gastroenteritis in Europe and specific agents may cause more severe disease in susceptible hosts. Based on the criteria for selection and prioritization, climate change related food- and water-borne pathogens were selected for this study. Because of their differences in behaviour in the environment, at least one pathogen of each of the groups of micro-organisms viruses, protozoan parasites and bacteria was selected. The selected pathogens are *Salmonella*, *Campylobacter*, *Cryptosporidium*, norovirus, *Vibrio*, *Listeria monocytogenes* (Table 1) and their characteristics are described in this chapter.

Table 1 Anticipated climate changes and the foreseen affected processes per selected pathogen (adapted from Lot 1).

Change in:	Salmonella	Campylobacter	Crypto.	Norovirus	Vibrio	Listeria
Temperature	growth, survival	survival	survival	inactivation	growth, survival	growth, survival
Extreme precipitation	water contamination	water contamination	water contamination	water contamination	growth, survival	water contamination
UV light	survival	survival	survival	inactivation	growth	survival
Drought	no effect	survival	survival	inactivation	no effect	survival
Salinity	no effect	no effect	no effect	no effect	growth	no effect
Relative humidity	survival, growth	survival, growth	survival	inactivation	no effect	survival

2.1 Norovirus

Noroviruses, belonging to the family of the *Caliciviridae*, are 28 – 35 nm in size, are non-enveloped viruses and contain a positive sense, single stranded RNA genome of approximately 7.6 kb (Green et al., 2002). The norovirus genus is divided into five genogroups (genogroup GGI to GGV). The diversity of norovirus variants increases continually due to the generation of new variants, and variants within GII.4 predominated in Europe in the past five years (Hohne and Schreier, 2004; Lopman et al., 2004; Lindell et al., 2005; Maunula and Von Bonsdorff, 2005; Reuter et al., 2005; Kroneman et al., 2008). Most human pathogenic noroviruses cluster within genogroup I (GGI) and genogroup II (GGII). No zoonotic potential could be demonstrated for these two genogroups, and are therefore considered to be solely human.

Noroviruses are the most common cause of food-borne viral gastroenteritis (Koopmans and Duizer, 2004). Norovirus infections cause acute gastroenteritis in humans. Symptoms include projectile vomiting, watery non-bloody diarrhea with abdominal cramps and nausea within 24 to 48 hours after exposure (McCarthy et al., 2000). Outbreaks have occurred in various settings such as nursing homes (Calderon-Margalit et al., 2005; Friesema et al., 2009), hospitals (Vinjé et al., 1997), cruise ships (Isakbaeva et al., 2005; Verhoef et al., 2008a), schools and universities (Kilgore et al., 1996), during pilgrimage (Verhoef et al., 2008b), restaurants and events with catered meals (Parashar et al., 1998). Transmission has occurred by person-to-person contact (Chadwick and McCann, 1994), through

contaminated water (De Serres et al., 1999; Hoebe et al., 2004) and food (Parashar et al., 1998; Pebody et al., 1998; Hutin et al., 1999). Most of the food items implicated in these outbreaks are eaten raw or uncooked such as oysters, mussels, fruits, vegetables, sandwiches, dairy products, baked products and salads leading to high risks of infection (Heller et al., 1986; Rosenblum et al., 1990; Le Guyader et al., 1994; Hutin et al., 1999; Daniels et al., 2000; Bosch et al., 2001; Potasman et al., 2002; Westrell et al., 2010). Foods are most likely contaminated through sewage contaminated surface water or by infected food handlers during harvesting, packaging or food preparation (Parashar et al., 1998; Daniels et al., 2000; Verhoef et al., 2010). Outbreaks originating from contaminated drinking water as well as from recreational water have been described (Hafliger et al., 2000; Boccia et al., 2002; Hoebe et al., 2004; Nygard et al., 2004).
Because no robust cell culture system for the detection of infectious human noroviruses is available (Duizer et al., 2004), information on the persistence of infectious virus particles in the environment is limited. Due to the non-enveloped structure of noroviruses, which is similar to those of other human enteric viruses, such as polio-, Coxsackie- and echovirus, noroviruses are presumed to be as resistant to environmental degradation and chemical inactivation as the other culturable human enteric viruses. Whether this stability is indeed comparable has to be studied, in the absence of an infectivity assay, by use of viral surrogates for human norovirus. At present, the most promising surrogate is the culturable murine norovirus due to its genetic similarity and environmental stability (Bae and Schwab, 2008). In general, infectivity reduction rates of surrogates were shown to be higher at higher temperatures (> 25ºC) and room temperature than at 4ºC as was studied for matrices such as surfaces of stainless steel, lettuce, berries, deli ham, surface and groundwaters (Cannon et al., 2006; D'Souza et al., 2006; Bae and Schwab, 2008; Butot et al., 2008). Also, the relative humidity is an important determinant for survival in the environment (Stine et al., 2005; Cannon et al., 2006). Data obtained by the stability of norovirus-like particles as well as surrogate viruses demonstrated stability over a pH range of 3 – 7 and up to 55ºC (Duizer et al., 2004; Ausar et al., 2006; Cannon et al., 2006).

2.2 Cryptosporidium

The intestinal parasite *Cryptosporidium* produces an environmentally robust oocyst to be able to endure environmental stress and to maximize the probability of reaching a new, susceptible host. *Cryptosporidium parvum* oocysts are spherical and about 3 to 5 µm in diameter. *Cryptosporidium* causes gastro-enteritis in humans; in otherwise healthy individuals, symptoms of *Cryptosporidium* infections generally persist for one to two weeks, but in immunocompromised person infections can be chronic with diarrhoea being severe and life threatening (Arrowood, 1997). At present, no consistently effective, approved therapeutic agent with anticryptosporidial activity is available.
Cryptosporidium oocysts are shed by infected persons or animals and enter surface water through direct fecal input, discharge of treated and untreated sewage, and runoff from agricultural lands; they are ubiquitous in surface water used for recreation or drinking water production. Oocysts are extremely resistant to chlorination at the concentrations commonly used for drinking water and swimming pool water disinfection.
Water-borne transmission of *Cryptosporidium* oocysts is associated with consumption of contaminated drinking water and recreation in contaminated surface water or swimming pools (Fayer et al., 2000). Many water related outbreaks of cryptosporidiosis have been reported over the past years (Fayer et al., 2004). Food-borne infections have been suggested to be caused by the consumption of contaminated school milk (Gelletlie et al., 1997), fresh-pressed apple cider (Millard et al., 1994; Blackburn et al., 2006), béarnaise sauce (Insulander et al., 2008) and salads (Besser-Wiek et al., 1996; Ponka et al., 2009), whilst a survey in Norway demonstrated the presence of *Cryptosporidium* on commercially available fruits and vegetables (Robertson and Gjerde, 2001).

2.3 Listeria monocytogenes

Listeria monocytogenes is a gram positive, non-sporeforming bacterium that can grow in anaerobic or aerobic conditions. Microscopically it appears as regular, short rods with rounded ends, 0.4-0.5 micrometer in diameter and 0.5-2 micrometer in length. It is present widely in the environment in soil, silage, (ground) water, sewage and decaying vegetation and may be part of the fecal flora of many mammals, including healthy human adults (Thevenot et al., 2006). Following ingestion by a susceptible human, the bacterium is capable of making the transition to a physiological state that promotes bacterial survival and replication in eukaryotic host cells (Freitag et al., 2009). In healthy individuals the disease caused by *L. monocytogenes* is usually restricted to a self-limiting gastroenteritis. However, in immunocompromised individuals, neonates and pregnant women, it is capable of causing systemic infections that lead to meningitis or encephalitis (Swaminathan and Gerner-Smidt, 2007). Also, in The Netherlands, the elderly have been reported to be predisposed to infection (Doorduyn et al., 2006). In pregnant women, infection of the developing foetus can lead to abortion, stillbirth or neonatal infections (Drevets and Bronze, 2008). Thirteen serotypes of *L. monocytogenes* have been identified, with three serotypes (1/2a, 1/2b and 4b) being associated with the majority of sporadic cases of listeriosis; serotype 4b is linked to almost all recent outbreaks (Thevenot et al., 2006).
The primary route of infection is by consumption of contaminated food products, but transmission by direct contact with contaminated products or surfaces has been described as well. *Listeria* bacteria have been isolated from a diverse array of products including dairy (cheeses, raw milk), agricultural (strawberries, cut fresh fruit, sliced mushrooms), and various ready to eat foodstuffs such as coleslaw, crab dip, smoked salmon and turkey and egg-, potato- and macaroni salad (Drevets and Bronze, 2008). L. monocytogenes grows at temperatures ranging from 0 to 45ºC and pH 4.1 – 9.6 and can survive in or on foods for long periods of time, possibly years. Freezing has little detrimental effect on the microbe. Although pasteurization is sufficient to inactivate *Listeria*, failure to reach the desired temperature in large packages can allow the organism to survive. Food can also be contaminated after processing by the introduction of unpasteurized material, and can be spread by contact with contaminated hands, equipment and counter tops (Bortolussi, 2008). Besides vertical transmission from mother to child, person-to-person transmission is very unlikely.

2.4 Vibrio

Vibrio species are gram-negative rods with a size of 1 micrometer in width and 2 to 3 micrometer in length and motile with at least one polar flagellum. They are common inhabitants of various aquatic environments. Many *Vibrio* species cause disease in aquatic animals such as fish, shellfish and marine mammals but also in humans (Oliver and Kaper, 1997). Some species, including *V. cholerae*, *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus* may cause disease in both aquatic animals and humans (Austin, 2010). Human pathogenic *Vibrio* species have been associated with wound infections (*V. alginolyticus*, *V. vulnificus*) and ear infections (*V. alginolyticus*) after exposure to contaminated waters, and gastroenteritis (*V. parahaemolyticus*, *V. cholerae* non-O1/O139) after consumption of contaminated food. More serious complications such as septicaemia (*V. vulnificus*) have also been reported, although these are rare and mainly occur in people who are immunocompromised or have a chronic liver disease (Oliver and Kaper, 1997; Morris, 2003). *V. cholerae* O1/O139 can cause acute watery diarrhoea in humans, which can lead to death, if left untreated (Morris, 2003).
Depending on the species, *Vibrio* bacteria tolerate a range of salinities and are common in marine environments with *Vibrio alginolyticus* being the most tolerant to high salinities and *Vibrio cholera* to low salinities. They are capable of multiplication in marine water at elevated water temperatures (>17-

20°C) (Morris, 2003). When environmental conditions are unfavourable, *Vibrio* species enter a viable non-culturable state which enables them to survive such conditions.

2.5 Campylobacter

Campylobacter bacteria belong to the family of *Campylobacteriaceae*. They are gram-negative, spiral-shaped, micro-aerophilic bacteria with either uni- or bi-polar flagella and are 0.2 - 0.5 by 0.5 to 5.0 micrometer in size. They are enteric commensals of many wild and domestic animals (cattle, sheep, waterfowl, poultry, pigs, and reptiles). The most common human pathogens among the more than 20 *Campylobacter* species are *C. jejuni*, *C. coli* and *C. lari*. Poultry is mostly colonised by *C. jejuni*, pigs by *C. coli* and waterfowl by *C. lari* (Blaser, 2000). In humans, campylobacteriosis is most commonly caused by *C. jejuni*. It produces an inflammatory, sometimes bloody diarrhea, mostly including cramps, fever and pain. Symptoms typically last for one week and disease is in most cases self-limiting. Less frequently, campylobacteriosis may result in more severe disease with joint inflammations, meningitis or Guillain-Barré syndrome. Campylobacteriosis affects people from all age groups, but is more frequently seen in infants under the age of six and young adults (Young et al., 2007).
Campylobacteriosis is among the most common bacterial infections in humans and often a food-borne illness, with chicken meat being the most frequent causative agent. Meats, including beef, pork and lamb have been implicated in infection as well. Consumption of vegetables and raw milk are not usually associated with infection although some cases have been described (Heuvelink et al., 2009). Surface water may become contaminated with *Campylobacter* by wild birds, domestic animals or sewage effluent, leading to exposure of humans to this bacterium (Murphy et al., 2006). A recent study on Dutch campylobacteriosis cases and controls indicated that risk factors for this disease may differ depending on age, season and degree of urbanization (Doorduyn et al., 2010). On a larger scale, such as the European territory, similar differences may exist between regions or member states.
Unlike other food-borne pathogens, *Campylobacter* spp. are fragile organisms that are unable to multiply outside the animal host or to grow in the presence of air. They are highly susceptible to a number of environmental conditions, such as UV, acidity, heat and cold stress. It has been reported that *C. jejuni* survives better at 4 °C in various biological environments than at 25 °C. *Campylobacter* spp. have been shown to enter a viable but nonculturable (VBNC) state when subjected to adverse conditions, such as low nutrient availability or upon entry into stationary phase, which is favourable for survival outside the host (Murphy et al., 2006).

2.6 Salmonella

Salmonella are rod-shaped, flagellated, gram-negative, facultative anaerobic bacteria with diameters around 0.7 to 1.5 µm, lengths from 2 to 5 µm, and flagella which project in all directions. *Salmonella* is a member of the family of *Enterobacteriaceae*. The genus *Salmonella* is divided into two species, *S. enterica* and *S. bongori*, of which the first species mostly affects humans. The best known *Salmonella* are *S. typhi* and *S. paratyphi*, the etiologies of enteric fever. However, the largest burden of *Salmonella* infections in industrialized countries is due to the nontyphoidal serotypes *S.* Enteritidis and *S.* Typhimurium (Crum Cianflone, 2008; Gantois et al., 2009). Furthermore, *Salmonella* are increasingly important because of the high incidence rates of infections worldwide, and the evolution of multiresistant strains.
The most common clinical manifestation of nontyphoidal salmonellosis is gastroenteritis, with symptoms as nausea, vomiting, and diarrhea occurring 6-48 hours after ingestion. The disease is usually self-limited after three to seven days, but the elderly and immunocompromised host have

higher risks for severe manifestations like bacteremia or endovascular complications (Crum Cianflone, 2008). In the EU, *S.* Enteritidis and *S.* Typhimurium are the serovars most frequently associated with human illness in 2008. The cases caused by *S.* Enteritidis are associated mostly with consumption of contaminated poultry meat and eggs, whereas cases caused by *S.* Typhimurium are associated mostly with consumption of pig, poultry and bovine meat (EFSA, 2010). Eggs and egg products are the most often identified food vehicles in *Salmonella* outbreaks, and >80% of all egg isolates were found to be *S.* Enteritidis (Gantois et al., 2009). According to several studies, the capacity to persist and grow in laid eggs is specific to serotype Enteritidis (Gantois et al., 2009). Also food items that are not directly derived from animals can be contaminated with *Salmonella*. The latter group includes for instance bean sprouts (Van Duynhoven et al., 2002). Food products can be contaminated intrinsically, with Salmonella multiplying within the product, or food can be contaminated with e.g. irrigation water. Furthermore, contaminated water is also associated with human infections, albeit less commonly (Angulo et al., 1997). Contamination of water may occur through fecal pollution by carriers. Aquatic vertebrates, such as birds and reptiles, are important polluters of water with *Salmonella*, but also poultry, cattle, and sheep can pollute water with Salmonella.
Salmonella bacteria can survive in a dry environment for long times, they have been found in dried excrement over a period of 2.5 years (Stine et al., 2005). They are frequently found in polluted water. Compared to other bacteria, *Salmonella* has high survival rates in aquatic environments and in soil and sediment, where they can survive and multiply for at least 1 year (Winfield and Groisman, 2003).

3 Sources for food and water-borne pathogens

Food- and water-borne pathogens may cause a public health threat from exposure to pathogens in water and food matrices. Sources for food- and water-borne pathogens can be humans, animals or environment. Except for the human noroviruses, the human pathogens selected for this study can infect humans and animals (Table 2). (Note that infection does not necessarily progresses to disease. Poultry can become infected with e.g. *Campylobacter*, leading to a colonised bird that is not observably diseased.) *Listeria monocytogenes* and *Vibrio* largely originate from the environment. Besides these two environmental agents that can replicate in water and food, also *Salmonella* is able to multiply outside the gut or intestines of its host. The other selected pathogens are enteric pathogens only replicating in the gut and the intestines of their specific hosts. Therefore, their transmission routes may be different though overlapping.

Table 2 Hosts, and possibility of multiplication in the environment, for the selected food- and water-borne pathogens.

	Host		Multiplication in the
Pathogen	Human	Animal	environment
Norovirus	yes	no	no
Cryptosporidium	yes	yes	no
Listeria monocytogenes	yes	yes	yes
Vibrio	yes	yes	yes
Campylobacter	yes	yes	not likely
Salmonella	yes	yes	yes

3.1 Water

Water-borne diseases are caused by ingestion of water that is contaminated by human or animal feces or urine containing pathogenic bacteria, viruses or parasites (Bradley, 1977). For the selected pathogens in the current project (see Chapter 2), contamination of both drinking water and recreational water are relevant.

3.1.1 Drinking water

Drinking water may be produced from source waters as diverse as groundwater, surface water (fresh- and seawater) and rainwater. Groundwater and fresh surface water are the major sources. The water may subsequently be treated or not and distributed through piped or non-piped supplies (WHO, 2008). For purposes of this report, the focus lies with fresh surface water and groundwater.

3.1.1.1 Groundwater

Groundwater is water located beneath the surface in pores and fractures with larger deposits being called an aquifer. The depth at which soil pore spaces or fractures and voids in rock are completely saturated with water is called the water table. Groundwater is recharged from, and eventually flows to, the surface naturally. Groundwater wells may produce drinking water. The integrity of the wells and the type of soil in which the well is situated largely contribute to the protection of the groundwater from contamination originating from human and animal sources. Except for *Vibrio*, each of the selected pathogens may be of concern for contamination of groundwater wells.

3.1.1.2 Surface water

Surface water is water collecting on the ground or in a stream, river, lake, wetland, or ocean. Surface water is naturally replenished by precipitation and naturally lost through discharge to evaporation and sub-surface seepage into the groundwater. Fresh water is used commonly for the production of drinking water. Following on collection the fresh surface water is generally treated with a train of treatment processes such as filtration and disinfection processes because of the high probability of pathogen contamination. Pathogens of concern may be any of the six selected pathogens.
Seawater may be used as a source for drinking water production but needs to be desalinated before use. The applied desalination processes are generally rigorous and likely to remove pathogens.

3.1.1.3 Rainwater

Alternatively, rainwater can be collected for use as a drinking water source. Harvesting and storage are critically important in the production of water of acceptable quality. Rainwater may be contaminated with e.g. the selected pathogens *Campylobacter* and *Cryptosporidium* from for instance birds (Schets et al., 2010).

3.1.2 Recreational water

3.1.2.1 Bathing sites in surface water

According to the revised European Bathing Water Directive (2006/7/EC) bathing sites in surface water are defined as any element of surface water where the competent authority expects a large number of people to bathe and has not imposed a permanent bathing prohibition, or issued permanent advice against bathing. These include sites that have officially been designated as bathing sites where water quality is checked on a regular basis for fecal indicator parameters *E. coli* and intestinal enterococci using methods specified in the Directive; results of these controls are reported to the European Commission. The Directive also applies to bathing sites that have not been designated as official sites but are visited by large numbers of bathers; water quality is monitored and competent authorities often consider these sites as future official bathing sites. Finally, there are bathing sites in surface water that are (infrequently) visited by small groups of bathers. These are no official sites, they are not under consideration to become official sites and water quality is not checked. Each of the selected pathogens may contaminate recreational waters.
Bathing sites differ in the composition of the water as well as in the environmental conditions, such as water temperature and sunlight irradiation. An apparent distinction in composition is based on the salt concentration, with for instance salt levels <0.5‰ for fresh water and ≥34.5‰ for seawater. Both fresh water and seawater recreation have been associated with increased health risks among the recreants (e.g., Schets et al., 2008; Stone et al., 2008). Enteric pathogens can be introduced into these waters by e.g. wastewater discharge or runoff from agricultural land, whereas other pathogens, like *Vibrio* spp. and *Pseudomonas* aeruginosa, are indigenous to many ecosystems. The fate and behaviour of both groups of pathogens depends on the composition of the surface water and the environmental conditions. Survival conditions in seawater are suboptimal for enteric pathogens, such as norovirus, *Campylobacter* and *Salmonella* due to the high salinity. Most *Vibrio* species, on the other hand, thrive well in the marine environment and some, like *V.* alginolyticus and *V.* parahaemolyticus, are associated with health complaints due to salt water recreation (Andersen, 2006; Schets et al., 2006). Some *Vibrio* species prefer a lower salinity and are therefore associated with health complaints due to fresh water recreation (*V.* cholerae non-O1/O139, *V.* vulnificus) (Oliver and Kaper, 1997; Motes et al., 1998). Due to different pathogen populations in marine and fresh water and the different effect that these environments may have on survival and/or proliferation of pathogens, the health risks for humans exposed to pathogens in these environments vary.

3.1.2.2 Natural or green pools

Outdoor pools with a separate swimming and filtration zone, are either situated in natural ponds, or use a concrete construction (old conventional pool or newly constructed) or PVC foil to form a pool. The filtration zone is planted with aquatic vegetation specially chosen to reduce levels of nutrients; in this zone organic matter and micro-organisms are trapped. There are drains to collect the water from this zone before it is pumped to the swimming zone. At this point sand filters and UV disinfection may be incorporated. The swimming zone is free of vegetation and from this zone the water flows into the filtration or regeneration zone. No disinfectants are used. Each of the selected pathogens may contaminate natural or green pools as outlined above in 3.1.2.1.

3.1.2.3 Paddling pools

Paddling pools are small pools with limited depth (0.2-0.6 m), commonly located outdoors in an urban environment and mainly used by children. These pools may become polluted very quickly because of the small water volume and the high bather load. Moreover, children may urinate or defecate in them and introduce pollution such as mud and grass from the surrounding area. Also, dogs and other animals may enter and pollute the pools. Ideally these pools are small enough to empty and clean every day. Safe water quality can be maintained by continuous circulation and simple filtration, with continuous chemical dosing. Paddling pools may be prone to fecal contamination with any of the selected pathogens.

3.1.2.4 Interactive water features and decorative fountains

Interactive water features are arrangements of ground sprays or jets, usually outdoors, designed to be used by children mainly. The design of these interactive features is different from that of decorative water features such as municipal fountains that are not designed for interaction although they are sometimes played in. In interactive water features, the sprays are installed in a surround, like stone or rubber, and the water usually drains through the surround into a holding tank from which the water is pumped to the sprays. Micro-organisms may be introduced on feet or other contacts that may transmit fecal contamination. Disinfection and filtration of the water should be applied to maintain microbiologically safe water.

3.1.2.5 Indoor pools

Swimming pools include conventional pools (by tradition rectangular) which may be located indoors or outdoors, but also hot tubs, spa pools, hydrotherapy pools etc. The source water is tap water and some form of pool water treatment is in place in all pool types. Commonly a residual disinfectant is maintained in the pool water to safeguard microbiological quality. In several European countries legislation is in place that requires the regular examination of swimming pool water for various microbiological and chemical parameters for which guideline or imperative values are specified in national legislation or standards.

3.2 Food

Food may become contaminated with pathogens either extrinsically on their surface from human or animal sources, intrinsically by infection of the production animal or passively by passage through and accumulation in the digestive tract. Food may be categorised into food groups, i.e. shellfish, dairy, fruits and vegetables, meat, drinks and grain (Rutjes et al., 2006). In each of the production chains primary production is followed by processing, retail and preparation.

3.2.1 Shellfish

Shellfish may be harvested from wild habitats or alternatively from seeded banks in culturing waters. Depending on the harvesting water quality shellfish may need to undergo depuration before they are packaged and prepared for consumption. Often shellfish are eaten raw (e.g. oysters) but they may be grilled or cooked (e.g. mussels). Shellfish may be contaminated with any of the selected pathogens, especially different *Vibrio* spp., if culturing waters in which the shellfish filterfeed are contaminated.

3.2.2 Dairy

Animal milk is mostly produced by cows or goats, but also by buffalo, sheep, horses or camels. Different products e.g. cheese, yoghurt, cream, butter *etc*. are produced from milk through several processes such as pasteurisation and sterilisation. Milk and milk products may be contaminated due to intrinsic infection of the production animal with e.g. *Campylobacter*, *Salmonella*, *Listeria* and *Cryptosporidium*. Furthermore, (re)contamination of products with pathogens can occur during processing, at retail or during preparation. EU-wide, less than 0.1% of milk samples collected at retail and cheeses collected at processing or retail were found to be contaminated with *Salmonella* (EFSA, 2010).

3.2.3 Fruits and vegetables

Vegetables may become intrinsically contaminated with pathogens via pathogen intake through its roots, as shown for Salmonella in experiments (Franz et al., 2007). Furthermore, extrinsic contamination of fruits and vegetables can occur in greenhouses or in the field due to exposure to potential faecal contamination sources, *e.g.* food handlers, animals, manure and irrigation water. Also during harvesting, processing and retail, dependent on good agricultural practices (GAP), the fresh products may be exposed to contamination with the selected pathogens. Especially noroviruses have been described as contaminant of berries and leafy greens. On the one hand, the pathogens may increase during the farm-to-fork trajectory due to recontamination or growth. On the other hand, the enteric pathogens may become inactivated. However, in case of fresh products, the farm-to-fork trajectories are relatively short. Consequently, pathogen growth and inactivation rates may be low, especially when products are stored at frigid temperatures. To reduce contamination levels, washing of products is sometimes applied. However, some products cannot be washed or treated because they are too fragile. Unpackaged products are more prone to recontamination in (super)markets.

3.2.4 Meat and eggs

Meat can be derived from slaughter of extensively or intensively farmed livestock and wild animals Meat may be sold as such, or processed into different meat products including sausages, cured ham and pate. Meat will be packaged in the meat industry or locally at retail. Animals may be intrinsically infected, possibly leading to contaminated meat and products. For *Salmonella*, poultry and pigs are the predominant animal reservoir, with an estimated ~3% of broiler flocks (EFSA, 2010), 6% of laying hen flocks (EFSA, 2010) and 10% of slaughter pigs (EFSA, 2009) being tested *Salmonella*-positive. Fresh broiler meat in Europe was contaminated in 2008 with *Salmonella* at a percentage of ~5% (EFSA, 2010). Furthermore, *Salmonella* was found in ~0.5% of the examined table eggs (single eggs or batches) and 1.1% of egg products (EFSA, 2010). For *Campylobacter*, poultry are the predominant animal reservoir, with an estimated 25% of broiler flocks comprising colonised broilers (n = 10,147) for seven EU member states that collected these data (EFSA, 2010).

3.2.5 Drinks

Drinks cover a wide range of liquid foods from mineral water and juices to alcoholic beverages produced from a variety of sources (milk is covered in 3.2.2 Dairy). Fruit juices such as derived from

apple, carrot, grape, have been implicated in outbreaks due to processing from faecally contaminated fruits and vegetables (e.g. Vojdani et al., 2008; Noel et al., 2010). Also, apple cider is prone to contamination with for instance *Cryptosporidium* (Blackburn et al., 2006).

3.2.6 Grain

Granary products may be complex such as sandwiches with butter, lettuce, egg and cheese. The grain source may be faecally contaminated with any of the selected enteric pathogens, but processing is likely to inactivate the contaminating pathogens. Alternatively, the sandwich filling may be faecally contaminated either during production or by food-handling.

4 Climate factors and food- and water-borne disease

Climate changes will affect the introduction, spread and growth of pathogenic micro-organisms in water and food, and therefore possibly also the number of infections, diseases and deaths by food- and water-borne pathogens (McMichael et al., 2001; Hunter, 2003). Exposure of humans (and animals) to food- and water-borne pathogens may take place either through consumption or by direct contact with contaminated sources. Food- and water-borne disease may occur through consumption of unboiled drinking water (produced from ground- or surface water) as well as through consumption of raw or undercooked food such as fruits, vegetables, shellfish and meat. Exposure by direct contact occurs mostly during recreational activities, through contact with soil or fresh water or seawater. Pathogen numbers and infectivity will be affected by climate factors resulting in die-off or inactivation or alternatively survival and growth of the pathogens. And therefore, any climate *change* may alter the resultant infectious disease burden from exposure to these pathogens.

Major climate factors that determine the increase or decrease of infectious micro-organism concentrations and thus may impact on food- and water-borne diseases include temperature, precipitation patterns, water availability and droughts. Other important environmental conditions are the availability of nutrients and minerals, pH, ionic strength and ion composition, moisture content and UV/sunlight (Bates et al., 2008). The impact of climate change on the diverse transmission routes of food- and water-borne diseases is depicted as a graphical conceptual model in Figure 1.

In the aquatic environment, the presence of pathogenic micro-organisms is much affected by the movements of the water itself. Such movements entail transport of micro-organisms from one water compartment to the other, for example runoff of manure from land to surface water, resuspension of pathogens from river sediments and dilution of water bodies.

With *rainfall*, pathogenic micro-organisms of human and animal faecal origin, here norovirus, *Cryptosporidium*, *Listeria monocytogenes*, *Campylobacter* and *Salmonella*, may enter surface waters by discharges of raw and treated wastewater and by runoff of faecal matter from wildlife, e.g. birds, waterfowl, deer, or domestic animals or manure from the land (Figure 1). This may lead to increased pathogen concentrations in shellfish, and irrigation and recreational waters. Alternatively, rainfall may lead to dilution of pathogen loads in aquatic environments or wash-off of pathogens from fruits and vegetables. Groundwater wells may be flooded due to rainfall leading to pathogen ingress into the wells. In contrast, the selected pathogens in general are resistant to drying, which likely minimizes the inactivation due to drought. *Relative humidity* influences the survival of the selected pathogens, as described in Chapter 2, in general at higher humidity pathogens survive better. *Sunlight* may indirectly enhance growth of bacteria, such as *Vibrio*, by raising water temperatures. In general, the selected pathogens are sensitive to UV inactivation.

In the production of drinking water and food, surface water, groundwater, crops, and animals are largely processed under controlled conditions. Obviously, under condition-controlled production processes climate factors do not play any role in the fate and behaviour of the pathogens. These controlled conditions for instance include storage of food products in a refrigerator or the rearing of broilers under controlled indoor climate conditions.
In contrast, storage, distribution and marketing of food products under non-controlled conditions are affected by climate conditions. Similarly, storage reservoirs used for reduction of water-borne

pathogens is an example of a step in drinking water production that is still subject to climate conditions. Also, drinking water in the distribution network may largely be exposed to climate conditions.

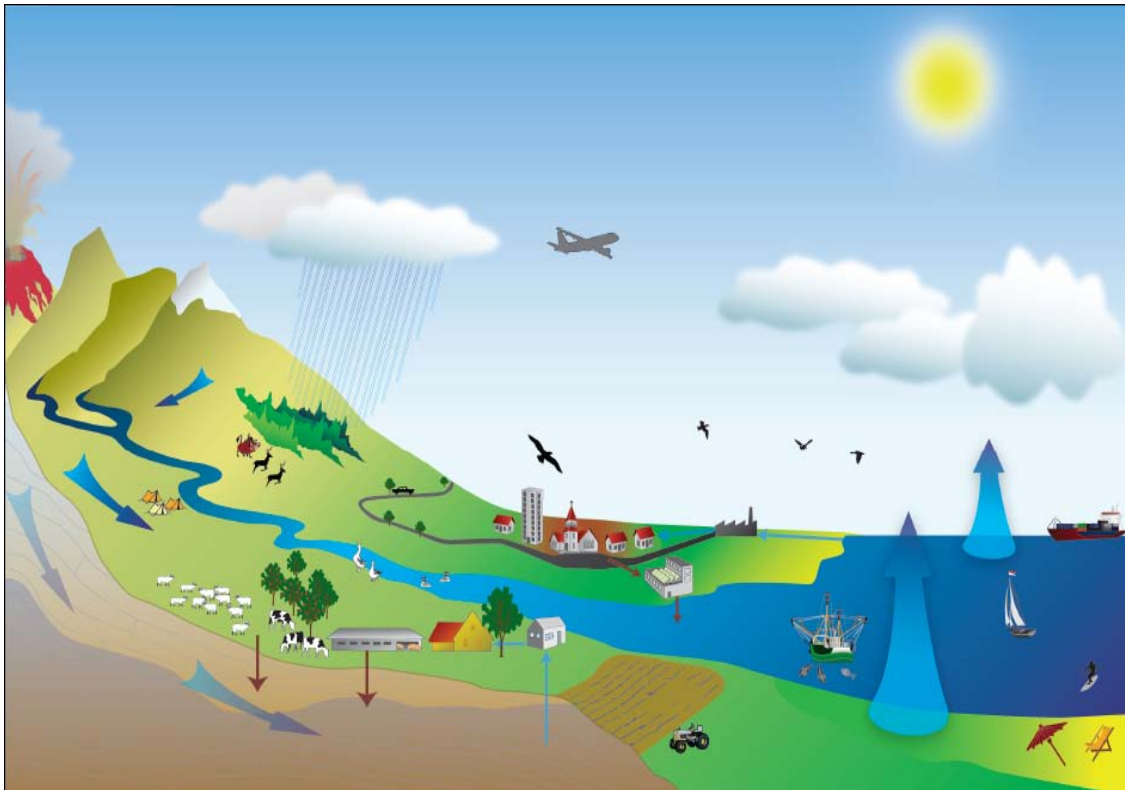

Figure 1 Graphical representation of the conceptual model for the impact of climate change on food- and water-borne disease

4.1 Impact of climate changes on food- and water-borne pathogens

The IPCC report of Bates et al. (2008) reports observed and projected changes in climate as they relate to fresh water. Table 3 lists the changes that affect the fate and behaviour of pathogens in the environment, supplemented with the pathogens that are anticipated to be affected by the climate change.

4.1.1 Temperature increase

Seasonality of water-borne pathogens has been well-reported. Commonly concentrations of enteric water-borne pathogens in river waters are higher in winter and lower in summer, mainly due to effects of temperature and residence times (Schijven and de Roda Husman, 2005). Especially higher water temperatures have a significant impact on the concentrations of water-borne pathogens in surface waters. In summer, temperatures are higher and residence times longer, therefore, enteric pathogens that cannot grow in the river water, such as noroviruses and *Campylobacter*, die-off or inactivate faster, whereas in winter they survive longer. Inactivation by increased water temperatures may be less for oocysts of *Cryptosporidium*, because they are less temperature sensitive and also more persistent

Table 3 Effect of climate change on the environment, fate and behaviour of the selected pathogens and the pathogens anticipated to be affected by the respective fate and behaviour.

Climate change	Environmental effect	Pathogen fate and behaviour	Affected pathogens*
Temperature increase	Water temperature increase	Increased growth of indigenous water-borne bacteria	Vi
		Increased inactivation/die-off of enteric water-borne pathogens	No, Sa, Ca, Cr
	Seasonal shift in water temperature and water flow	Seasonal shift in concentrations of water-borne pathogens in surface water	No, Sa, Ca, Cr, Vi
	Temperature conditions of food on markets, farms	Increased growth of foodborne pathogens	Vi, Li, Sa, Ca
		Increased inactivation/die-off of food-borne pathogens	Cr, No, Vi
Rainfall intensity and frequency	Flooding Run-off Resuspension of river sediments Sewage overflows	Increase in intensity and frequency of peak concentrations of water-borne pathogens in surface water	All
	Groundwater table changes	Increased contamination of groundwater from faecal sources	All
		Increased remobilisation of deposited pathogens	Cr, No, Sa, Ca
Water availability and drought	Change of water source Atmospheric water vapour content Snow cover and melting of ice	Change in source water quality (pathogen concentrations)	All
	Soil moisture	Change in growth of food- and water-borne pathogens	Vi, Sa, Ca
		Change in inactivation/die-off of food- and water-borne pathogens	No

* Ca: *Campylobacter*; Cr: *Cryptosporidium*; Li: *Listeria*; No: norovirus; Sa: *Salmonella*; Vi: *Vibrio*.

(Schijven et al., 1996). Concentrations of *Listeria*, *Vibrio* and to a lesser extend *Salmonella* may increase in certain circumstances, due to increased temperatures. In storage reservoirs the effects of increased temperatures may be even stronger, because of longer residence times (Schijven and de Roda Husman, 2005). Because warmer years are expected to occur more often, indigenous water-borne pathogens, such as *Vibrio* spp. may have more opportunities to grow. As a consequence, there may be an increased exposure to these kinds of pathogens during recreational activities (Schijven and de Roda Husman, 2005).

The effect of increased inactivation due to higher temperatures may be overshadowed by peak concentrations from runoff and sewer overflows following heavy rainfall events (Schijven and de Roda Husman, 2005). The occurrence of peak concentrations has been reported, but is difficult to predict (Westrell et al., 2006). Since peak concentrations determine the risk of infection, this effect is likely to be of more importance than enhanced inactivation.

Concerning temperature increase and food-borne transmission of pathogens, the control of cold chains may be impeded by rising ambient temperatures. Humidity may increase production of mycotoxins, whereas certain food-borne pathogens may thrive better under warm conditions. Seasonality of *Salmonella* spp. in dairy cows (Pangloli et al., 2008) or gilt (Vonnahme et al., 2007) has been reported. This seasonality is partially caused by the birth season. A shift and altered duration of this season may be caused by changing temperatures due to climate change. Furthermore, increased ambient temperatures have been suggested to increase *S. Enteritidis* growth in products containing raw eggs (Van Pelt et al., 2004). Although the incidence of salmonellosis in general decreases, studies concerning food-borne disease showed an increase in the number of salmonellosis cases of 5-10% at every 1 ºC weekly increase in temperature (Kovats et al., 2004).

4.1.2 Rainfall intensity and frequency

If, as a consequence of rainfall, runoff increases, concentrations of enteric pathogens in fresh water increase. Water-borne pathogens, such as *Cryptosporidium*, may also be resuspended from river sediments (Rose, 1998), leading to peak concentrations, which strongly affect associated health risks (Schijven and de Roda Husman, 2005). Therefore, shifts in amplitude and timing of concentrations of water-borne enteric pathogens may occur.

It may be assumed that runoff increases proportionally with increases in extreme precipitation (Kok et al., 2002). This implies that peak concentrations of water-borne pathogens in surface water as a consequence of precipitation are likely to occur increasingly more in high latitudes (Northern Europe), but not in the subtropical and lower mid-latitude regions (Southern Europe). This indicates an adverse effect of precipitation leading to peak concentrations of pathogens in surface water, but a beneficial effect where there is less precipitation and therefore longer residence times. These impacts of precipitation may vary strongly between regions in Europe.

Rain-generated floods may carry water-borne pathogens to land and thereby increase the probability of exposure to these pathogens; in addition, such floods may also pick up water-borne pathogens from agricultural land. Flooding may lead to increased contamination of crops in the field, or increased exposure of food animals to zoonotic agents. Furthermore, precipitation and flooding may impact groundwater quality.

4.1.3 Water availability and drought

Because of the significant decrease in water storage in mountain glaciers and Northern hemisphere snow cover, shifts in the amplitude and timing of runoff in glacier- and snowmelt-fed rivers and lakes, have been observed (Bates et al., 2008). Therefore, shifts in amplitude and timing of concentrations of water-borne enteric pathogens may likely be the case. Ground water tables may be lowered due to drought or elevated due to increased precipitation, but an effect on the risk of groundwater contamination with water-borne pathogens is not clear.

Adaptation options designed to ensure water supply during average and drought conditions require integrated demand-side as well as supply-side strategies. The former improve water-use efficiency, e.g., by recycling water. Such developments may impact the microbial quality of source waters, in those cases where water management practices are affected. Also, one may need to rely on lower quality water because of water scarcity. Recycling of water requires sufficient treatment of water to restore its microbiological quality.

Water availability may or may not be indirectly associated with the microbiological quality of the water. Scarcity or loss of water sources may lead to the use of source water of less quality, with increased risks and higher demand on treatment. The use of water of less microbiological quality for irrigation may lead to increased contamination of crops in the field by enteric pathogens.

4.2 Data needs

In order to predict the fate and behavior of water-borne pathogens as a consequence of climate changes, primarily data on their growth rates and inactivation or die-off rates under the various environmental conditions are required. Such data are at least in part available in literature (see for example chapter 2).

5 Quantitative risk assessment modelling tools

5.1 Risk assessment framework

Quantitative microbiological risk assessment (QMRA) is used to estimate adverse health events from exposure of individuals to a hazard, such as a pathogen (Haas et al., 1999; Vose, 2000). To this end, pathogen concentrations in the source are estimated. Subsequently, changes in the pathogen concentration during processing (*e.g.*, slaughter) or treatment (*e.g.*, for drinking water production) are modelled to estimate an individual risk of an adverse health event such as infection or illness. A general framework to conduct QMRA is available, with four steps to be considered (Figure 2) (ILSI, 1996). Firstly, the problem at hand is defined and the potential risks and exposure routes are identified (hazard identification). Secondly, the exposure to the pathogen for individuals is quantitatively estimated, yielding for instance an estimated ingested dose of the pathogen (exposure assessment). Thirdly, a dose-response model is obtained, either from literature or from experimental data depending on the availability, which describes the relation between a dose being ingested and the probability of developing an adverse health event. And fourthly, the estimated dose from the exposure assessment is related to the dose-response model to characterize the risk of infection. This general framework can also be used for evaluating the effect of climate change on microbiological health risks, but some modifications are required as described below.

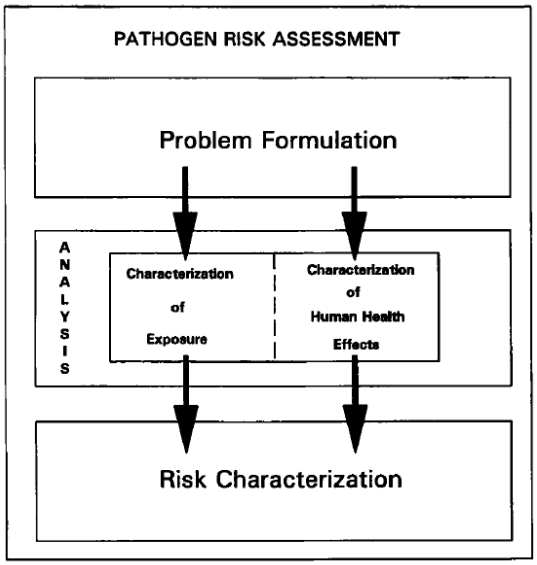

Figure 2 The conceptual framework for risk assessment according to ILSI (1996)

The exposure assessment within the general framework can be conducted in various ways. One such way is the modular process risk model (MPRM) (Nauta, 2008). In its origin, the MPRM is a QMRA tool that provides a modular structure for transmission models of food production chains. The tool can take into account the variability and uncertainty around the parameters describing the transmission pathway. Each MPRM module is a mathematical description of a process that relates a pathogen input to a pathogen output. Combining successive modules gives a description of the transmission pathway and can be used to estimate an ingested dose. The original MPRM approach comprises seven steps (Nauta, 2008): 1) defining the statement or purpose of the model, 2) describing the food pathway, 3) building the MPRM model structure by splitting up the pathway into the modules, 4) collecting the available data and expert opinions according to the model structure developed, 5) selecting the mathematical model to be used for each module, 6) feeding the available data into the model, and 7) assessing the exposure.

As stated, the MPRM was originally developed to model the transmission of bacteria through the food chains. Its concept, however, is well applicable to other areas. This statement applies also to climate change, where different regions experience different effects of climate change. By having available an electronic library of modules that describe the theoretically possible effects of climate change on the presence of water- and foodborne pathogens, region-specific exposure assessment models can be constructed by combining modules as applicable.

In addition to the modifications of the MPRM approach to develop the tool, the general QMRA framework needs to be modified to estimate a relative risk due to effects of climate change. This requires the modules to be designed to enable both a description of the current state (the baseline) as well as an effect of health risks due to anticipated local climate change(s). The current QMRA concept will be modified within this project such that a change in risk due to climate change can be estimated.

5.2 Required modules

As explained, the QMRA tool will consist of separate modules that describe the fate and behaviour of the six selected micro-organism during their transmission from environmental sources to humans. These modules can be connected according to specific situations to estimate the effect of climate change on public health risks for specific regions.

Figure 3 shows the concept of an MPRM with one module in a chain of three as an example. The depicted module describes mathematically the inactivation of *Campylobacter* spp. in water as function of the change in temperature. Required input parameters are the concentration of *Campylobacter* spp. in water, the current water temperature, the anticipated water temperature due to climate change, the thermal decay rate of *Campylobacter* spp. and the time period that is taken into consideration. Output of the module will subsequently be the increased or reduced concentration of *Campylobacter* spp. per liter of water due to the change in water temperature.

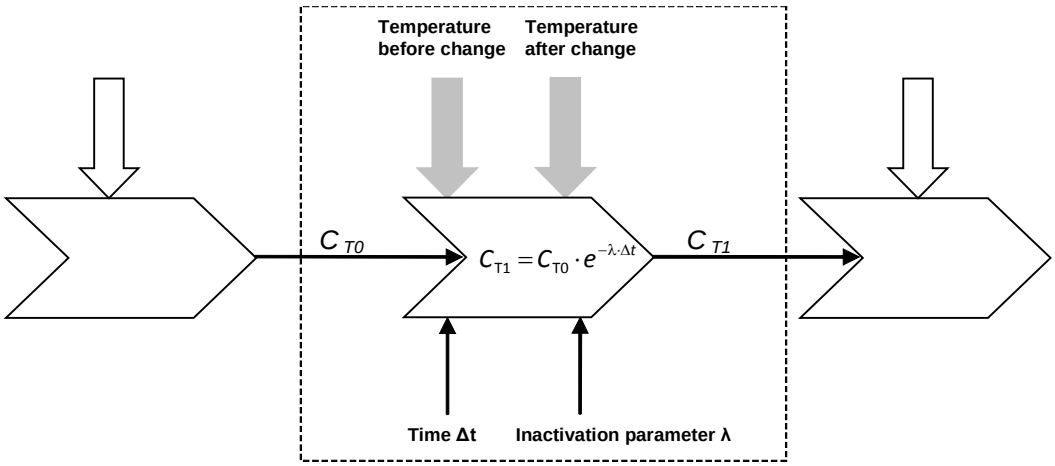

Figure 3 Example of a module (within the dashed square) for the effect of temperature change on the Salmonella spp. concentration in water and its linkage to other modules

Examples of modules that will be developed within this project are listed in Table 4. These modules describe the change in growth or die-off of bacteria due to the change in temperature. Another anticipated climatic change involves the precipitation, which is anticipated to change in intensity as well as frequency. This change may lead to a higher probability of flooding or run-off of pathogens from surfaces such as land, possibly increasing the contamination of the environment and therefore human exposure to pathogens. Furthermore, residence times may shorten or lengthen due to the change in precipitation, influencing *e.g.* the thermal inactivation by affecting the parameter 'time' in the respective module. Another anticipated climate change, albeit highly uncertain (Bates et al., 2008), is solar exposure, possibly affecting the exposure of pathogens to UV. Other processes for which the effects of climate change are less clear, will also be examined for relevance and feasibility of translation into modules. These processes include drinking water treatment processes for disinfection and removal of pathogens and a change in evaporation due to the temperature change.

Table 4 Modules that describe the effect of temperature change on the presence of pathogens

Module	Explanation
Bacterial growth in water	Depending on the temperature increase, bacteria grow faster or slower
Thermal decay/inactivation in water	Depending on the temperature increase, bacteria and viruses decay faster or slower
Bacterial growth on surfaces	Depending on the temperature increase, bacteria grow faster or slower
Thermal decay/inactivation on surfaces	Depending on the temperature increase, bacteria and viruses decay faster or slower

6 Tool description

The CCMQMRA tool consists of 22 modules, subsets of which can be linked consecutively to estimate the relative infection risks for 13 pathogen-pathway combinations. This chapter contains the mathematical background of each of the modules, and explains how data should be entered in the tool and how results should be interpreted.

6.1 Outline

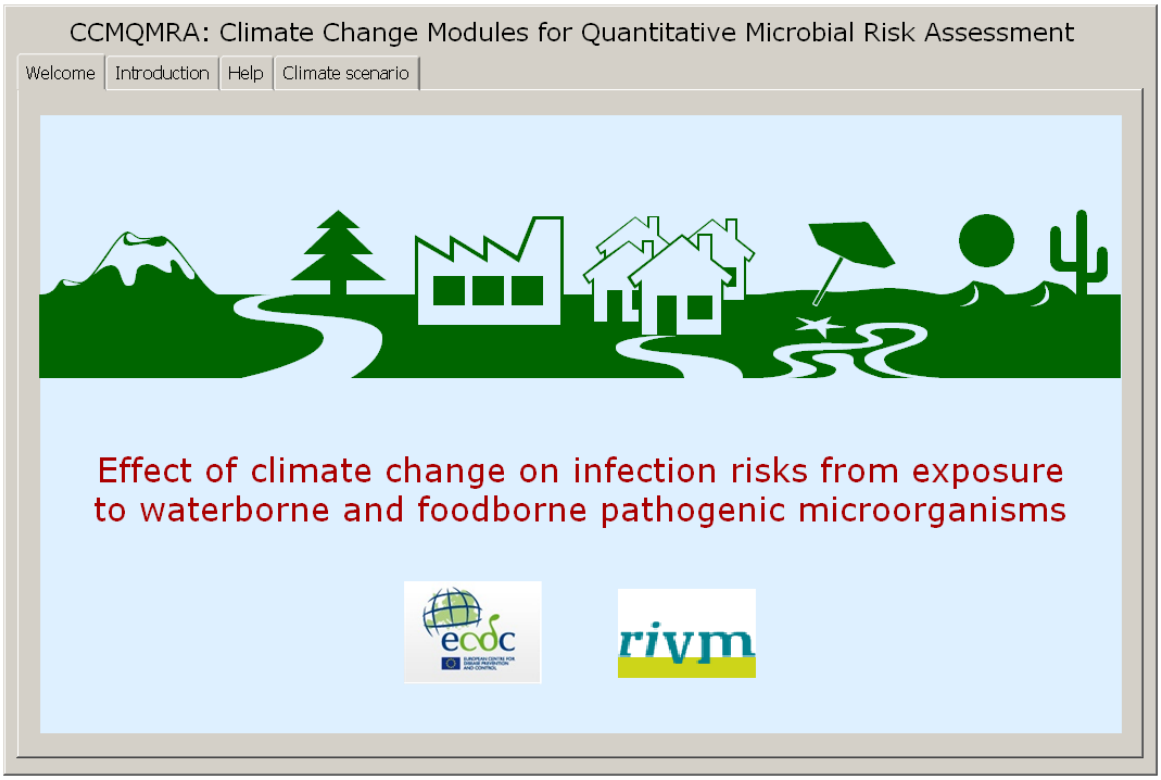

Figure 4 CCMQMRA Welcome screen

CCMQMRA: Climate Change Modules for Quantitative Microbial Risk Assessment is an interactive computational tool to calculate effects of climate change on infection risks from exposure to water-borne and food-borne pathogenic micro-organisms. It is programmed in Mathematica 7 (Wolfram Inc) and converted to run in Mathematica Player 7 (which is freely available from Wolfram Inc).
The 'Introduction'-tab (Figure 4) provides information on the objectives of the tools and the target group of policy makers, environmental engineers and scientists.
The output of the tool is an estimate of the relative infection risk for a particular pathogen-pathway combination (the pathways being drinking water, surface water, chicken filet, oysters or eggs). The effects of climate change on infection risks are determined by comparing calculated infection risks under current and future climate conditions.

The ‘help’-tab contains a glossary and help information on all parts the tool, and can be consulted during the use of the tool as user manual. The help information entails information on how to use the tool and to enter data, as well as technical background information on the models that are used. Under the Climate scenario tab, current and future climate conditions are defined, encompassing air and water temperatures, annual precipitation and number of heavy rainfall days.
Under the ‘Pathogen pathway’-tab, the pathogen of interest and the matrix (or pathway) can be selected. Having made these selections, a risk assessment (QMRA) model is constructed automatically by linking the required modules for this pathway. The next step requires data entry by the user for each of the modules by going through the modules step-by-step (each module is represented by a tab). The order by which modules are available and updated is automatically controlled by the tool.
In addition to the ‘Help’-tab, help information is available on each screen. The majority of items are accompanied by a so-called tool-tip: additional information is shown when the mouse is moved over the item.

6.2 Climate scenario

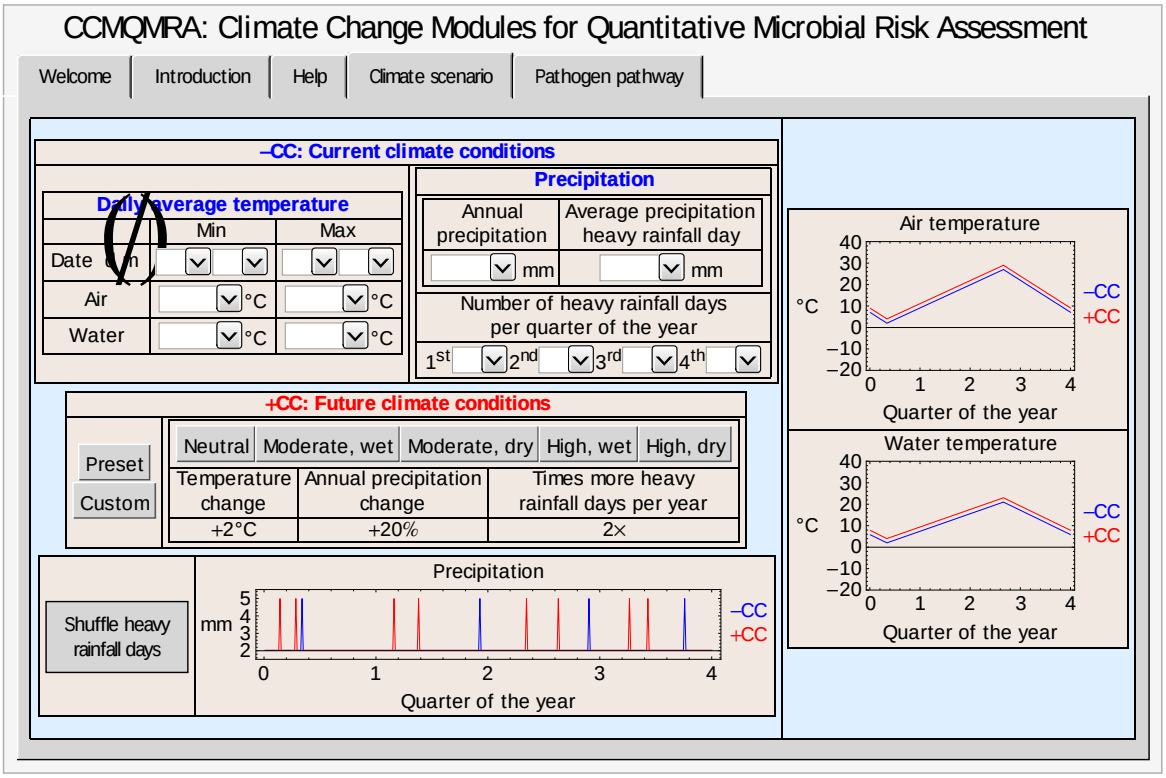

Figure 5 Climate scenario screen

In the Climate scenario screen (Figure 5), current and future climate conditions can be set. These conditions can for instance be obtained from a meteorological institute. The meaning of ‘future’ is defined by the user. For example, it can be over twenty years, or the next century.
Current climate conditions that can be set are: daily average air and water temperatures, annual precipitation, average precipitation on a heavy rainfall day and the number of heavy rainfall days per

quarter of the year. Throughout the tool ‘CCMQMRA’, current climate conditions are indicated in blue and labelled ‘-CC’ (minus CC) and future climate conditions in red labelled ‘+CC’ (plus CC).
Future climate conditions can be set as temperature change, change in annual precipitation and the multiplication of more heavy rainfall days per year. A choice can be made from five preset scenarios, or values can be defined individually (custom). The values that can be selected encompass IPCC scenarios, and can be location-specific. A table with the chosen climate change is shown as reminder on each screen of the tool.
Heavy rainfall days are randomly distributed for each quarter of the year. By pressing the Shuffle heavy rainfall days, the heavy rainfall days are randomly redistributed.
The right half of the screen shows the air and water temperatures for each day of the year under current (blue) and future (red) climate conditions. Average daily temperatures are assumed to gradually increase from the coldest to the warmest day and then gradually decrease.

6.3 Pathogen pathway

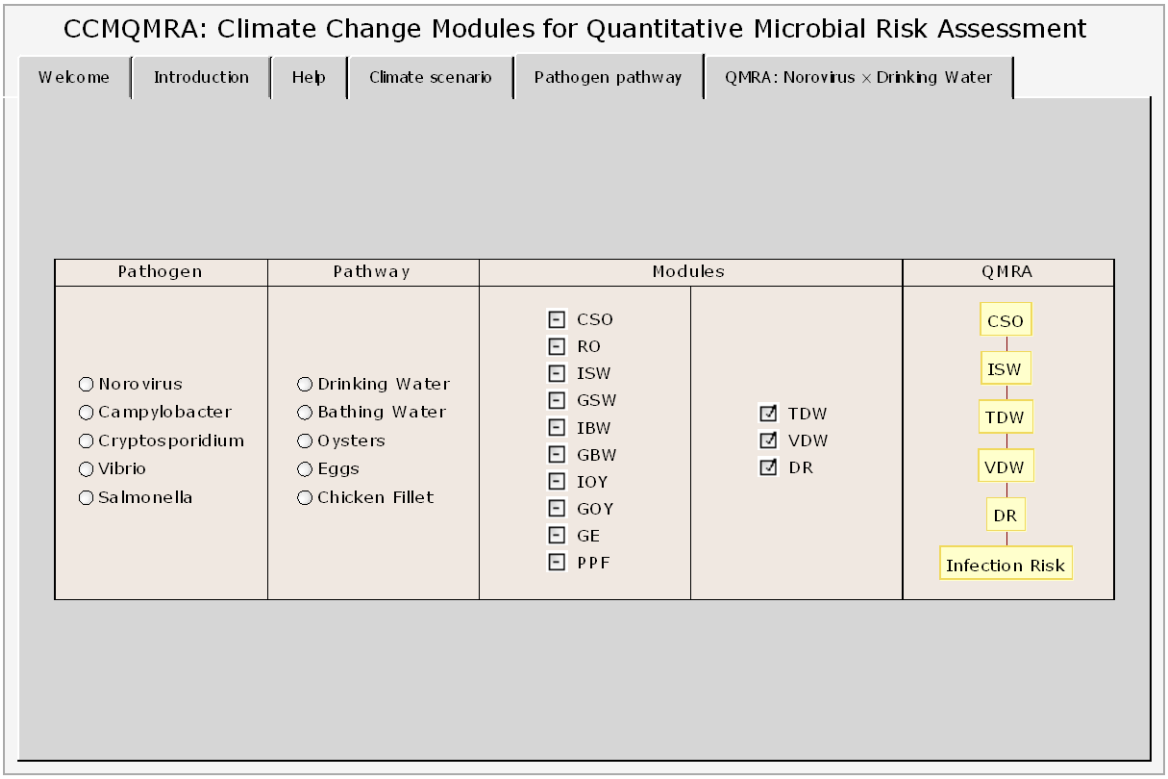

Figure 6 Pathogen pathway screen

The pathogen pathway screen (Figure 6) lists the pathogens and pathways that can be selected.
For each pathogen-pathway selection, a number of modules is pre-selected. If the selection square is white, then it can be (de)selected. If grey, then it cannot be changed. The latter aspect of the tool prevents estimating relative risks for non-realistic combinations of pathogens and pathways.
At the far right a so-called QMRA-tree is shown, given the order of the selected modules in the QMRA. This QMRA-tree also becomes visible when the mouse goes over the ‘QMRA: pathogen × pathway’-tab. In total, there are thirteen such QMRA combinations available (Table 5), consisting of a

selection of the 22 modules available. The modules for treatment of drinking water (TDW), consumption (VDW, VBW, COY, CCF, CE), dose response (DR) and risk of infection are always combined on one QMRA screen. CCMQMRA is a highly versatile tool, because the thirteen QMRA combinations can be combined with various location-specific climate conditions and climate changes.

Table 5 Possible Pathogen pathway selections

Pathogen	**Pathway**	**Modules**
Norovirus	Drinking water	CSO→ISW→TDW→VDW→DR→Risk of infection
	Bathing water	CSO→IBW→VBW→DR→Risk of infection
	Oysters	CSO→ISW→IOY→COY→DR→Risk of infection
Campylobacter	Drinking water	CSO*→RO*→ISW→TDW→VDW→DR→Risk of infection
	Bathing water	CSO*→RO*→IBW→VBW→DR→Risk of infection
	Oysters	CSO*→RO*→ISW→IOY→COY→DR→Risk of infection
	Chicken fillet	PPF→CCF→DR→Risk of infection
Cryptosporidium	Drinking water	CSO*→RO*→ISW→TDW→VDW→DR→Risk of infection
	Bathing water	CSO*→RO*→IBW→VBW→DR→Risk of infection
	Oysters	CSO*→RO*→ISW→IOY→COY→DR→Risk of infection
Vibrio	Bathing water	GBW→VBW→DR→Risk of infection
	Oysters	GOY→COY→DR→Risk of infection
Salmonella	Eggs	GE→CE→DR→Risk of infection

*modules can be deselected. Other selections are fixed.

Table 6 Module names and abbreviations

Abbreviation	Module name
CSO	Combined Sewer Overflow
RO	Run Off from agricultural land
ISW	Inactivation in Surface Water
GSW	Growth in Surface Water
IBW	Inactivation in Bathing Water
GBW	Growth in Bathing Water
IOY	Inactivation in Oysters
GOY	Growth in Oysters
GE	Growth in Eggs
PPF	Prevalence in Poultry Flocks
TDW	Treatment of Drinking Water
VDW	Volume of unboiled Drinking Water
VBW	Volume of swallowed Bathing Water
COY	Consumption of Oysters
CCF	Consumption of raw/undercooked Chicken Fillet
CE	Consumption of raw/undercooked Egg(product)
DR	Dose Response
Risk of infection	Risk of Infection

6.4 Module CSO: combined sewer overflow

6.4.1 Pathogens

Norovirus, *Campylobacter*, *Cryptosporidium*

6.4.2 Mathematical description

The pathogens listed in 6.4.1 are excreted by infected humans and transported to a wastewater treatment plant by the sewerage system. By treatment of the wastewater at the wastewater treatment plant, concentrations of pathogen are reduced, commonly by a factor of 10 – 100 (1-2 $\log_{10}$ units). The treated wastewater is discharged onto surface water (river/stream/canal), resulting in dilution of pathogens depending on the size of the surface water. In combined sewers, the household wastewater is mixed with rainwater before reaching the wastewater treatment plant. In the case of a heavy rainfall event, the maximum capacity of the wastewater treatment plant may be reached leading to an accumulation of untreated sewage and sewers overflow. During this combined sewer overflow (CSO), untreated wastewater is discharged onto surface water, leading to peak concentrations of pathogens in the surface water. Depending on the efficiency of the wastewater treatment in removing pathogens,

these peak concentrations can be several orders of magnitude higher and therefore represent higher risk moments.
In a climate scenario with an increase in the number of heavy rainfall events, it is assumed that there is an equal increase in the amount of CSOs. If in the climate change scenario annual precipitation has increased, there is more dilution of the discharged wastewater in the surface water.
Thus there are days with and without combined sewer overflow for current and future climate conditions (indicated by indices 0 and 1, respectively), that are described by four equations to calculate the pathogen concentration in the surface water at the discharge point, C_{sw}. The parameters used in the following formulas are explained in Table 7

Current climate conditions, $365 - n_{peak}$ days no CSO:

$$C_{sw,0} = C_{in} Z_{wtp} \frac{Q_{in}}{Q_{sw}} \tag{5}$$

Current climates conditions, n_{peak} days with CSO:

$$C_{sw,0} = f_{in} C_{in} \left(Z_{wtp} + f_{out} \right) \frac{Q_{in}}{Q_{sw}} \tag{6}$$

Future climate conditions with $365 - f_{peak} \times n_{peak}$ days no CSO:

$$C_{sw,1} = C_{in} Z_{wtp} \frac{Q_{in}}{f_r Q_{sw}} \tag{7}$$

Future climate conditions with $f_{peak} \times n_{peak}$ days with CSO:

$$C_{sw,1} = f_{in} C_{in} \left(Z_{wtp} + r_{peak} \frac{365 - n_{peak}}{r_y - \sum r_{peak}} \right) \frac{Q_{wtp}}{f_r Q_{sw}} \tag{8}$$

Table 7 CSO module parameters

Parameter	Dimension	Symbol
Wastewater Treatment Plant		
Pathogen raw wastewater concentration	N/liter	C_{in}
Wastewater treatment	Log_{10}	$Log_{10} Z$
Wastewater treatment, standard deviation	Log_{10}	$StDev$
Discharge rate of treated wastewater	m^3/day	Q_{wtp}

Table 7 Continued.

Combined Sewer Overflow		
Change in pathogen raw wastewater concentration during CSO	N/liter	$f_{in}C_{in}$
Discharge rate of raw+treated wastewater	m^3/day	$r_{peak}\frac{365-n_{peak}}{r_y-\sum r_{peak}}Q_{wtp}$
Surface water (River/Stream/Canal)		
Flow rate	$10^3 \times m^3$/day	Q_{sw}
Width	m	w_{sw}
Depth	m	d_{sw}

6.4.3 Assumptions

All the values of the parameters in equations 5-8 are assumed to be constant, with the exception of the wastewater treatment $Log_{10}Z$. This parameter is assumed to have a normal distribution with standard deviation *StDev*. The wastewater treatment efficiency varies from day to day and therefore Monte Carlo samples are taken from the lognormal distribution to mimic this variation in the relative risk estimation. It is assumed that CSO occurs on each heavy rainfall day and that the discharge rate of raw plus treated wastewater is proportional to the increased precipitation on a heavy rainfall day.

6.4.4 How to enter data in the tool

Parameter values can be entered by choosing a number of preset values (for example, Low, Medium, High, or Small, Medium, Large) (Figure 7). These preset values are values taken from literature (Tables 8 and 9). Under 'Set'-buttons, it is possible to enter location-specific values. These data can for instance be obtained from managers of wastewater treatments plant. For the other part, a choice can be made from a range of values in a pull-down menu.

In the right half of the tool screen, a 'Do Monte Carlo' button can be pressed. Pressing this button induces calculation of the pathogen concentration in the surface at the point of discharge for current and future climate conditions using the values that are set in the left half of the tool screen. A graph appears that shows the pathogen surface water concentration for each day in the year. Each time a parameter value is changed the 'Do Monte Carlo' button reappears and needs to be pressed again to update pathogen concentrations. Furthermore, if parameter values in the 'Climate scenario' screen are altered, or if another selection is made in the 'Pathogen pathway' screen, then the 'Do Monte Carlo' button needs to be pressed again.

In the graph, pathogen concentrations are at two levels. The lower level being from discharge without CSO, varying from day-to-day. The higher level represents the CSO events that are concurrent with heavy rainfall events as set in the 'Climate scenario' screen. Current and future climate conditions are indicated in blue and red, respectively.

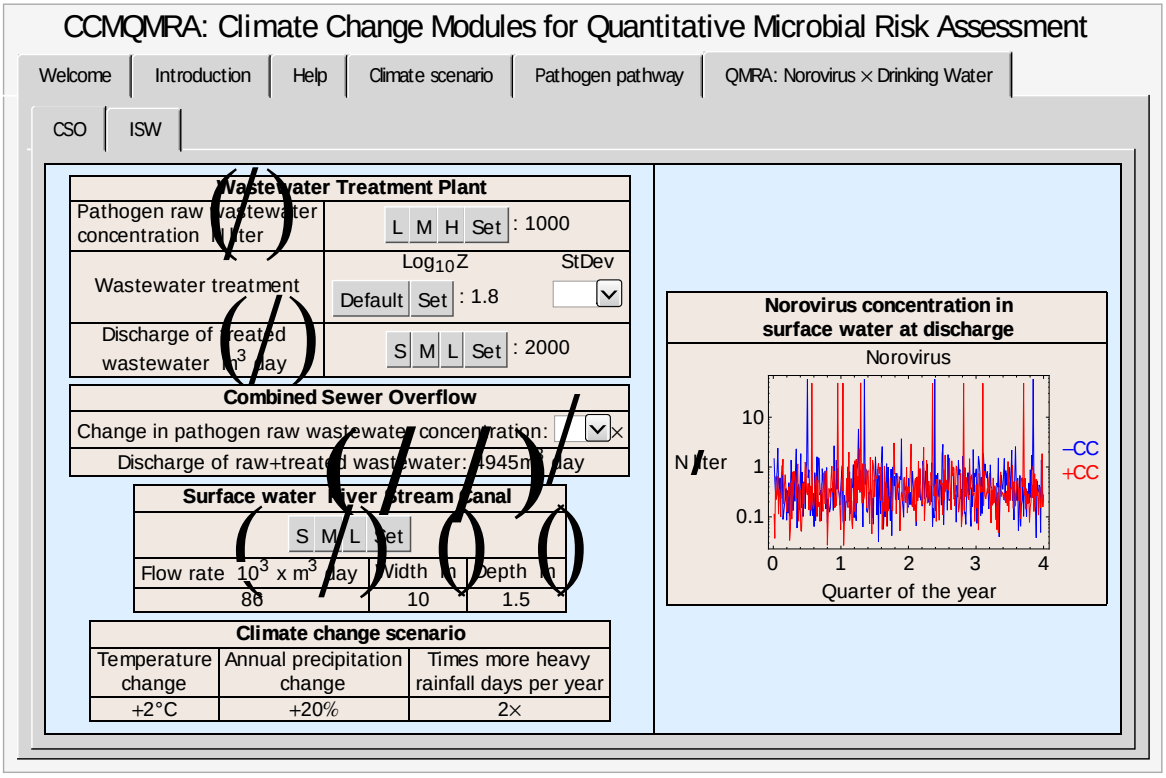

Figure 7 CSO: Screen image of Combined Sewer Overflow module

Table 8 CSO parameter values

Parameter	Dimension	Value	Reference
Wastewater Treatment Plant			
Pathogen raw wastewater concentration	N/liter	Low, Medium, High, Set	
Wastewater treatment	Log_{10}	Default from literature or range from 0-5	
Wastewater treatment, standard deviation	Log_{10}	Default from literature or range from 0- $Log_{10}Z / 2$	
Combined Sewer Overflow			
Change in pathogen raw wastewater concentration during CSO*	N/liter	0.1, 0.2, 0.5, 1, 2, 5, 10	
Discharge rate of treated wastewater	m^3/day	Calculated, proportional to heavy rainfall	
Surface water (River/Stream/Canal)			
Flow rate	$10^3 \times m^3$/day	86, 2200, 23000	Schijven et al. (2005)
Width	m	10, 50, 125	Schijven et al. (2005)
Depth	m	1.5, 2.6, 3.8	Schijven et al. (2005)

* In case these data are available. If not, then the default value of '1' can be used, indicating that the pathogen concentration in raw wastewater does not change during a CSO

Table 9 Influent concentrations and removal by wastewater treatment

	Influent concentration (N/liter)				
Pathogen	**L**	**M**	**H**	**$Log_{10}Z^*$**	**Reference**
Norovirus	1000	10000	100000	1.8	Lodder and De Roda Husman (2005)
Campylobacter	1000	10000	1000000	1.0	Havelaar (2001)
Cryptosporidium	100	1000	10000	1.4	Hoogenboezem et al (2000, 2001)

* *Z* represents the fraction of pathogens that passes treatment

6.5 Module RO: surface runoff of water from land into surface water

6.5.1 Pathogens

Norovirus, *Campylobacter*, *Cryptosporidium*

6.5.2 Mathematical description

Campylobacter and *Cryptosporidium* are so-called zoonotic pathogens, *i.e.* they can be transmitted from infected animals to humans. Manure on agricultural land contains such pathogens. Due to rainfall, they may be released from the manure and washed off the land and runoff to the surrounding surface water. The amount of runoff depends on soil-specific cover characteristics. In that regard, parameter S (from eqn. 9) represents the potential maximum retention for specific land covers and is related to soil-specific cover characteristics and is calculated from a so-called runoff curve number, CN , which is the volume of water that will run off per 100 cm^3 of water and has a value from 30 to 100. The value for the runoff curve number, *CN*, depends on several factors, including the hydrologic soil group (HSG), the type of ground cover, treatment, the hydrologic condition, the antecedent runoff condition (ARC), and whether impervious areas are connected to drainage systems, or whether they first outlet to pervious area before entering the drainage system. The U.S. Soil Conservation Service (1986) provides average *CN* values for different land types, averages of which are used as default values in the RO module.

The value for S can be calculated from:

$$S = \frac{1000}{CN} - 10 \qquad (9)$$

Table 10 Default CN values for different land uses and hydrological soil groups

		Hydrological Soil Group*			
		A	B	C	D
Grassland	<50% grass cover	68	79	86	89
	50%-70% grass cover	49	69	79	84
	>70% grass cover	39	61	74	80
Fallow	Crop residue cover	75	85	90	92
Row crops		66	76	82	85
Small grain		61	73	81	84

* A: <10% clay and >90% sand or gravel; B: clay between 10% and 20%, sand between 50% and 90%; C: clay between 20% and 40%, sand <50%; D: clay >40%, sand <50%, clayey textures (USDA, 2007).

To calculate the volume of runoff water, the equation given by the U.S. Soil Conservation Service (1986) is used:

$$RO = \frac{(r - 0.2S)^2}{r + 0.8S} \tag{10}$$

with RO being the amount of runoff (mm), r the amount of rainfall (mm) and S the potential maximum amount of water (mm) that will be retained by the soil, corrected for all losses before runoff begins (*i.e.*, water retained in surface depressions, water taken up by vegetation, and loss due to evaporation and infiltration).

Under current as well as future climate conditions, there are days with average precipitation and days with heavy rainfall, meaning there are three levels for r as defined in the"Climates scenario" screen.

Current climate conditions, $365 - n_{peak}$ days with average precipitation:

$$r_{low,0} = \frac{r_y - n_{peak} r_{peak}}{365 - n_{peak}} \tag{11}$$

Current climates conditions, n_{peak} days with heavy rainfall: r_{peak}

Future climate conditions with $365 - f_{peak} \times n_{peak}$ days with average precipitation:

$$r_{low,1} = \frac{f_r r_y - f_{peak} n_{peak} r_{peak}}{365 - f_{peak} n_{peak}} \tag{12}$$

Future climate conditions with $f_{peak} \times n_{peak}$ days with heavy rainfall: r_{peak}.

Substitution of the different r's into equation (10), gives three corresponding levels for RO: $RO_{low,0}$, $RO_{low,1}$ and RO_{peak}.

Runoff occurs from an area of agricultural land along the riverbank of length l_{rb} and width w_a. Pathogens that run off are diluted in the surface water. Thus there are days with and without heavy rainfall for current and future climate conditions (indicated by indices 0 and 1, respectively, that are described by four equations to calculate the pathogen concentration in the surface water along the river bank bordering agricultural land, C_{swro}.

Current climate conditions, $365 - n_{peak}$ days with average precipitation:

$$C_{swro,0} = C_{ro} \frac{l_{rb} w_a R0_{low,0}}{Q_{sw}} \tag{13}$$

Current climates conditions, n_{peak} days with heavy rainfall:

$$C_{swro,0} = C_{ro} \frac{l_{rb} w_a R0_{peak}}{Q_{sw}} \tag{14}$$

Future climate conditions with $365 - f_{peak} \times n_{peak}$ with average precipitation:

$$C_{swro,1} = C_{ro} \frac{l_{rb} w_a R0_{low,0}}{f_r Q_{sw}} \tag{15}$$

Future climate conditions with $f_{peak} \times n_{peak}$ days with heavy rainfall:

$$C_{swro,1} = C_{ro} \frac{l_{rb} w_a R0_{peak}}{f_r Q_{sw}} \tag{16}$$

Table 11 RO module parameters

Parameter	Dimension	Symbol
Run off curve number	-	CN
Volume of runoff water	m^3	$RO_{low,0}$, $RO_{low,1}$, RO_{peak}
River bank length	km	l_{rb}
Area width	m	w_a

6.5.3 Assumptions

All the values of the parameters in equations 10-16 are assumed to be constant. It is assumed that pathogen concentrations in run off water equal those in raw wastewater, but this assumption can be changed by pressing the ‘set’-button and entering a specific value (see section 6.4.4). It is assumed that the water from the whole area of agricultural land along the river ban k runs off to the river. Note that no literature data were found on run off concentrations of pathogens.

6.5.4 How to enter data in the tool

Parameter values can be entered by choosing a number of preset values (For example, Low, Medium, High, or Small, Medium, Large). These preset values are values taken from literature. Under “Set” buttons, it is possible to enter location-specific values when available. For the other part, a choice can be made from a range of values in a pull-down menu.
The size of the receiving surface water is the same as for CSO. In the case that CSO is included it is already defined in CSO, if not, it can be defined here as in CSO (see section 6.4.4).
In the graph, pathogen concentrations are at two levels. The lower level is from runoff during average daily precipitation. The higher level represents peak runoff on days with heavy rainfall as set in the “Climate scenario” screen. Current and future climate conditions are indicated in blue and red, respectively.

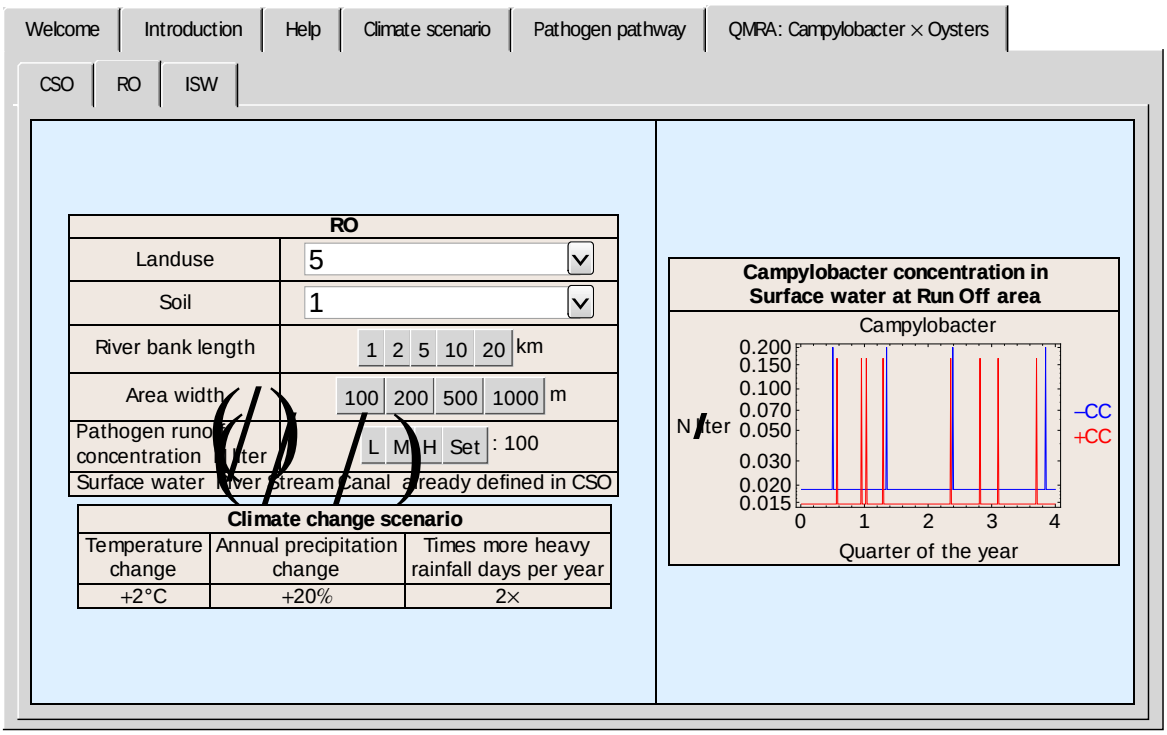

Figure 8 RO: Screen image of Run Off module

Table 12 Parameter values for the surface runoff module

Parameter	Dimension	Value	Reference
Landuse	-	List of types of crops, see Table 5	U.S. Soil Conservation Service (1986)
Soil	-	List of types of soil, see Table 5	U.S. Soil Conservation Service (1986)
River bank length	km	1, 2, 5, 10, 20	-
Area width	m	100, 200, 500, 1000	-
Pathogen runoff concentration			
Campylobacter	N/liter	1000, 10000, 1000000	Havelaar et al. (2001)
Cryptosporidium	N/liter	100,1000, 10000	Hoogenboezem et al. (2001)

6.6 Modules ISW and IBW: temperature dependent inactivation or die-off in surface/bathing water

6.6.1 Pathogens

Norovirus, *Campylobacter*, *Cryptosporidium*

6.6.2 Mathematical description

Enteric pathogens enter surface water by discharges of raw and treated wastewater, by runoff from agricultural land and may directly be coming from birds and wildlife. With the exception of some enteric bacteria under specific conditions, enteric pathogens that have entered surface water gradually inactivate or die-off. Inactivation or die-off rates are strongly dependent on the type of microorganism and proceeds faster at higher temperature. Also, this temperature dependent inactivation or die-off is microorganism-specific. Here, a first order temperature dependent inactivation or die-off rate is assumed:

$$C_{t,T} = C_0 EXP\left[-\frac{\ln 10}{10^{a_0 + a_1 T}}\right] t \qquad (17)$$

where C_0 is the initial concentration (N/liter), t is the time (days), T is the temperature (°C), a_0 ($\log_{10}$ day) and a_1 ($\log_{10}$ day °C^{-1}) are inactivation rate parameters, and where $10^{a_0 + a_1 T}$ is the time for one $\log_{10}$ reduction.

Between the source of contamination and the location where surface water is taken in for drinking water production, or where is a bathing area or oyster bank, there is a certain distance and travel time. After a travel time of n days, pathogen concentrations are reduced by inactivation or die-off. In the ISW and IBW modules, a time step of 1 day is used. Concentration $C_{n,T}$ is calculated as follows:

$$C_{n,T} = C_0 EXP\left[-\ln 10 \sum_{i=1}^{n} \frac{1}{10^{a_0 + a_1 T_i}} \right] \quad (18)$$

where T_i is the temperature (°C) on the *i*-th day.

6.6.3 Assumptions

First order rate inactivation is assumed. It is assumed that the $\log_{10}$ of the time to achieve one log reduction is linearly dependent on the water temperature.

6.6.4 How to enter data in the tool

Inactivation or die-off parameter values are provided by the module and are taken from the literature. The time 1 $\log_{10}$ inactivation at 10°C and 20°C is calculated and shown to demonstrate temperature-dependence. The module shows what sources of contamination are present, commonly wastewater discharge CSO) and/or runoff (RO). If these are absent, the module requires entering a local pathogen surface water concentration. CSO and RO can be at different distances from the point of drinking water intake/bathing area/oyster bank. The so-called travel distance can be entered for each those sources. The available choices are 1, 2, 5, 10, 20 and 50 km. The module calculates travel times from the travel distances and the river flow rate (as defined in CSO or RO). Dependent on the climate scenario for total annual precipitation, travel times between current and future conditions may be different. For example, if there is less precipitation under future conditions, there is less dilution of discharged wastewater in the surface water, hence a higher pathogen surface water concentration at that point. The lower precipitation also implies longer travel times. At the point of intake for drinking water/bathing area/oyster bank, pathogen surface water concentrations have decreased exponentially. In the particular case that the pathogen is sensitive to temperature and with sufficient time for inactivation, decrease can be so much higher under the future climate conditions, that future concentrations are now the lowest at the end-point. In the right half of the screen pathogen concentration for each day in the year and for current (blue) and future climate (red) conditions at the point of intake for drinking water production is shown. Especially for those pathogens that are temperature sensitive, like *Campylobacter*, concentrations are lowest, when water temperatures are highest.

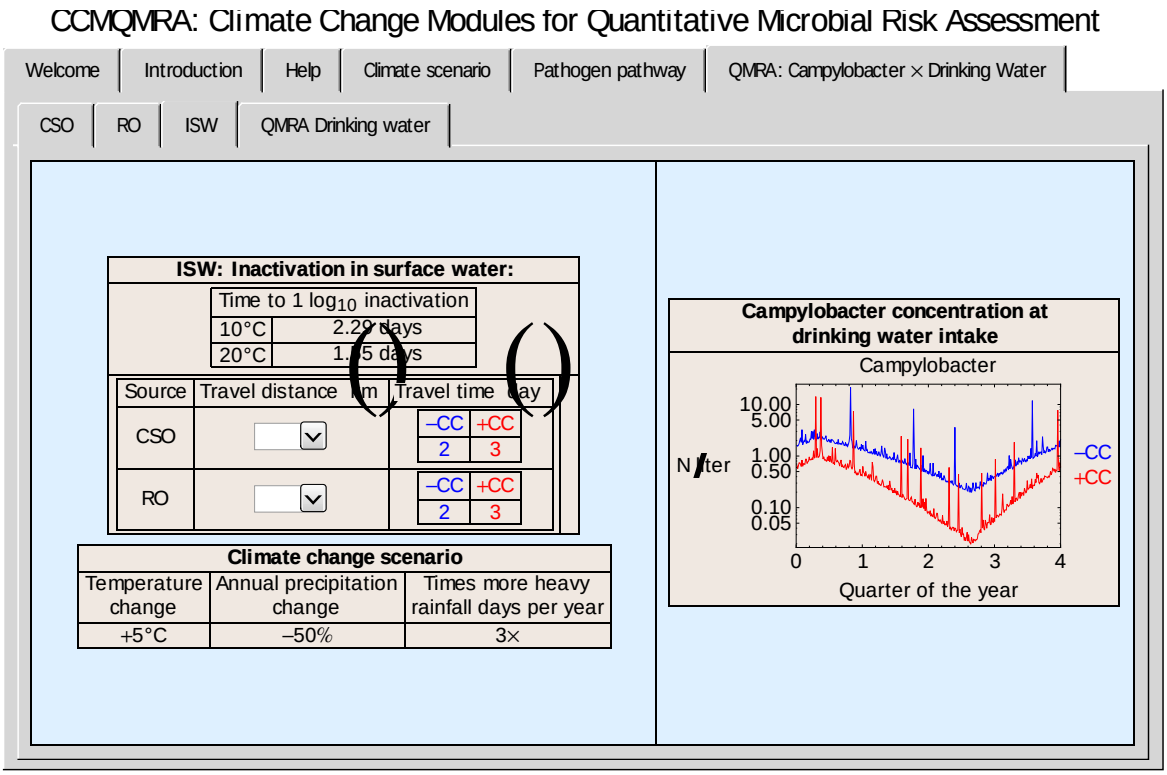

Figure 9 ISW: Screen image of Inactivation in Surface Water module (same for bathing water)

Table 13 Inactivation parameter values

Pathogen	Inactivation rate parameters		Reference
	a_0	a_1	
Norovirus	2.3	-0.035	Bertrand et al. (in prep.)
Campylobacter	0.53	-0.17	Havelaar (2001)
Cryptosporidium	3.1	-0.078	Ives et al. (2007)

6.7 Modules GSW and GBW: temperature dependent growth in surface/bathing water

6.7.1 Pathogens

Vibrio non cholerae species

6.7.2 Mathematical description

It is assumed that *Vibrio* at low temperatures is present at a minimum concentration. Above 17 – 20°C, rapid growth to a maximum concentration occurs. Minimum and maximum concentrations, as well minimum growth temperature depend on temperature, salinity and pH, and also on *Vibrio* species.

6.7.3 Assumptions

It is assumed that lag times for growth and inactivation can be neglected on the time scale of days.

6.7.4 How to enter data in the tool

Because of the simple model that is used, only minimum and maximum concentrations, and minimum growth temperature can be entered. The minimum concentration has a default value of 1 per liter, and can be set to 0.01, 0.1, 1, 10 and 100 per liter. The maximum concentration has a default value of 10^4 per liter, and can be set to 10^2, 10^3, 10^4, 10^5 and 10^6 per liter. The minimum growth temperature is by default 20°C based on the current literature, but can be set to 15 – 25°C when for instance results of future experiments show a different value. The right of the screen shows the *Vibrio* concentration in bathing water for each day of the year under current (blue) and future (red) climate conditions. The concentration varies between the minimum and maximum concentrations, depending on the water temperatures, hence the block shape of the curves. The period that concentration is at maximum is longer under future climate conditions because of temperature increase. The screen is the same for modules GBW and GSW.

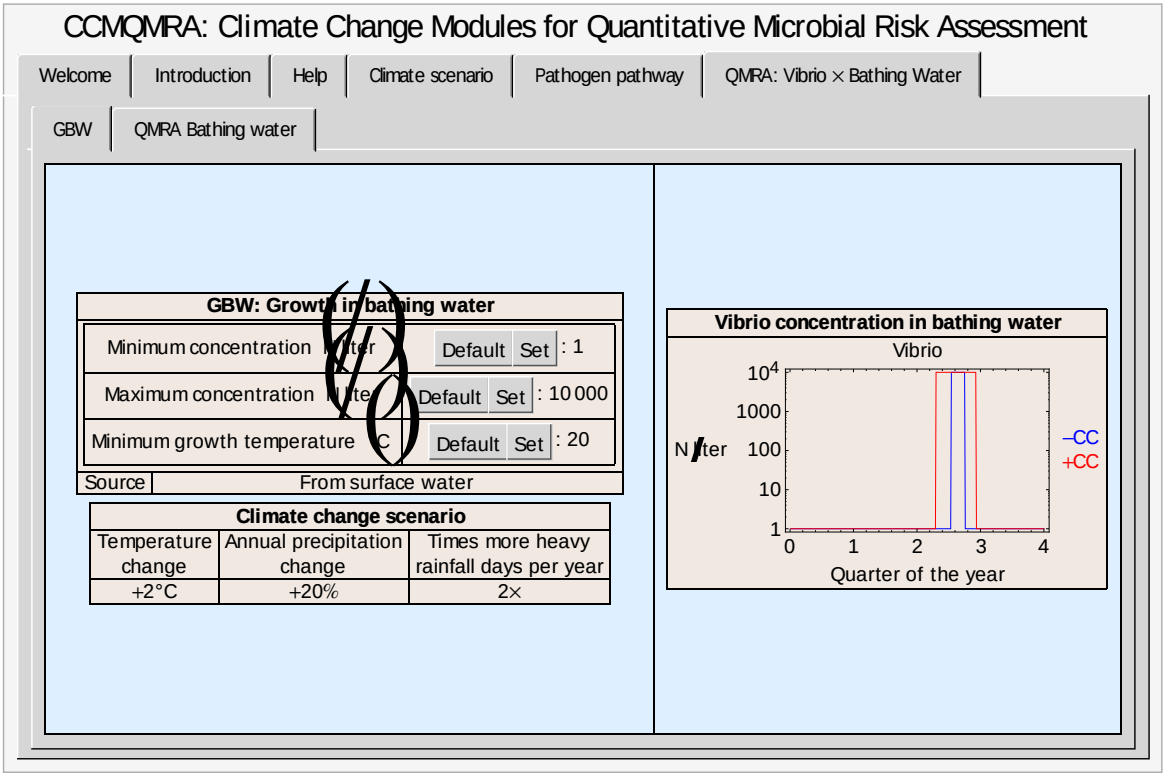

Figure 10 GBW: Screen image of Growth in Bathing Water module (same for surface water)

6.8 Module IOY: Temperature dependent inactivation or die-off in oysters

6.8.1 Pathogens

Norovirus, *Campylobacter*, *Cryptosporidium*

6.8.2 Mathematical description

The same model as described in section 6.4.2 is used (modules ISW and IBW).

6.8.3 Assumptions

See section 6.4.3. It is assumed that the same inactivation or die-off parameter values apply for inactivation or die-off in surface water, bathing water and oysters.

6.8.4 How to enter data in the tool

Inactivation or die-off parameter values are provided by the module and are taken from literature. The time 1 $\log_{10}$ inactivation at 10°C and 20°C is calculated and shown to demonstrate temperature-dependence. The source of contamination is surface water in which the oysters were cultivated. Because of filtration, pathogen concentrations in the oysters may be several times higher than in the surface water. This can be entered as a concentrating factor, and is very much pathogen specific. This concentration factor is currently not well estimated, and therefore no literature-based values can be entered. When such data become available, the concentrating factor can be changed to represent this value.

Oysters are transferred to water of lower faecal contamination for depuration, where during depuration inactivation takes place. Depuration time can entered: 0 – 7 and 14 days. In addition, dependent on the type of pathogen, pathogens may be washed out, but this aspect is currently not included in the tool due to a lack of data.

The right half of the screen shows the pathogen concentration in oysters after depuration for each day of the year for current (blue) and future (red) climate conditions.

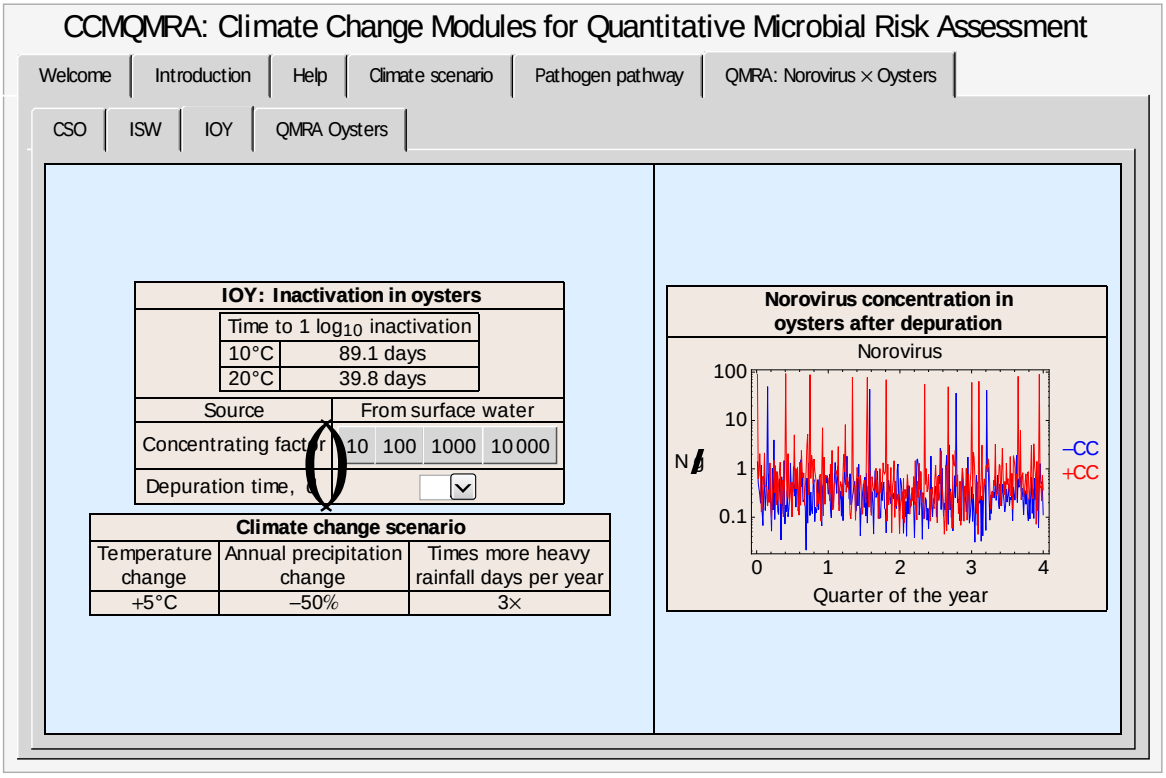

Figure 11 IOY: Screen image of Inactivation in Oysters module

6.9 Module GOY: Temperature dependent bacterial growth in oysters

6.9.1 Pathogens

Vibrio non cholerae species

6.9.2 Mathematical description

See section 6.5.2.

6.9.3 Assumptions

See section 6.5.3

6.9.4 How to enter data in the tool

Because of the simple model that is used, only minimum and maximum concentrations, and minimum growth temperature can be entered. The minimum concentration has a default value of 1 per liter, and can be set to 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} and 10 per gram. The maximum concentration has a default value of 10 per gram, and can be set to 0.1, 1, 10, 10^2 and 10^3 per gram. The minimum growth temperature is by default 20°C and be set to 15 – 25°C. Contrary to module IOY, depuration is not considered because of growth of *Vibrio*. The right of the screen shows the *Vibrio* concentration in oysters for each day of the year under current (blue) and future (red) climate conditions. The concentration varies between the minimum and maximum concentrations, depending on the water temperatures, hence the block shape

of the curves. The period that concentration is at maximum is longer under future climate conditions because of temperature increase.

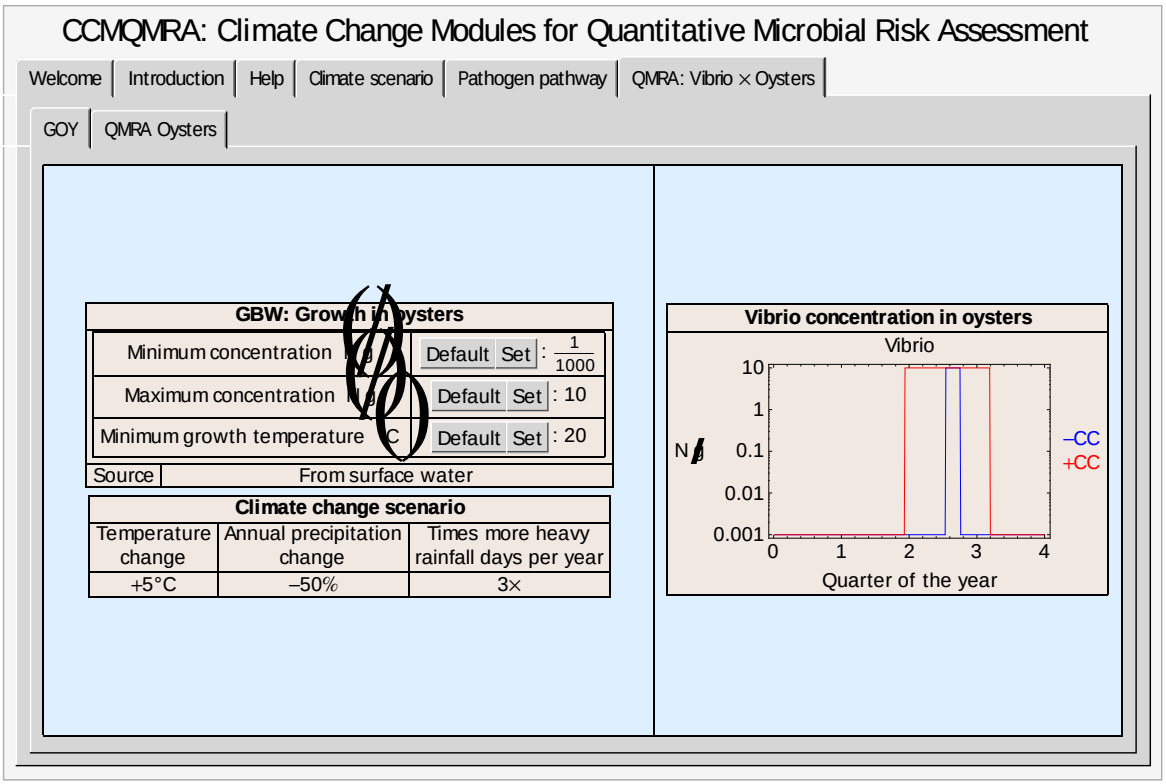

Figure 12 GBW: Screen image of Growth in Oysters

6.10 Module GE: growth of *Salmonella Enteritidis* in eggs

6.10.1 Pathogens

Salmonella Enteridis

6.10.2 Mathematical description

The risk associated with *Salmonella enteritidis* (SE) in eggs depends on the probability of having SE in an egg, and on the probability of SE growth in an egg. In order to grow in an egg SE first needs to migrate from the egg albumin through the vitelline membrane to the egg yolk. The default value for the probability of SE in an egg, p_{cont} originates from the EFSA Community Surveillance Report (EFSA, 2010), where 0.5% of 13659 samples were found positive for *Salmonella*. For the current study, it was assumed that all these detected *Salmonellae* were of the type e*nteritidis*. The probability of migration ($p_{migrate}$) was studied experimentally by Cogan et al. (2001) and found to depend on the concentration of *Salmonella* in the egg albumin, C_{low} (low indicates no growth). For a C_{low} value of 2 CFU/egg the probability was 0.066, for 25- 250 CFU/egg ~0.3, and for 2500 CFU/egg it was 0.5. These values have been used as default values in the tool. For intermediate values of C_{low}, the following empirical relation is used:

$$p_{growth} = 0.05 + 0.053 \log_{10} C_{low} \tag{19}$$

This implies that there are eggs in which SE has not grown (relatively low concentration of SE) and in which it has (relatively high concentration of SE), with the following probabilities of occurrence:

$$p_{low} = p_{cont}\left(1 - p_{growth}\right) \tag{20}$$

$$p_{high} = p_{cont} p_{growth} \tag{21}$$

The growth model that is used is a modification of the Baranyi and Roberts model in which the lag phase term was removed (Franz et al. 2010):

$$\begin{aligned} T > T_{\min} \quad & C_{t,T} = e^{\mu_{\max} t} \Big/ \left(\frac{1}{C_0} + \frac{1}{C_{\max}} \left(e^{\mu_{\max} t} - 1 \right) \right) \\ T \le T_{\min} \quad & C_{t,T} = C_0 \end{aligned} \tag{22}$$

with maximum growth concentration, $C_{\max} = 10^8$ CFU/ml (Grijspeerdt et al., 2005; Gumudavelli et al., 2007). Maximum growth rate $\mu_{\max}$ is calculated according to Gumudavelli (2007):

$$\mu_{\max} = 0.002065(T - T_{\min})^2 \tag{20}$$

where $T_{\min} = 6.13$ °C.

The concentration of SE, $C_{n,T}$, after n days of exposure to ambient air temperatures in a contaminated eggs, in which SE has entered the egg yolk, is calculated in n iterations using a time step of 1 day:

$$\begin{aligned}
&T_1 > T_{\min} \quad C_{1,T_1} = e^{\mu_{\max}} \Big/ \left(\frac{1}{C_0} + \frac{1}{C_{\max}} \left(e^{\mu_{\max}} - 1 \right) \right) \\
&T_1 \le T_{\min} \quad C_{1,T_1} = C_0 \\
&T_2 > T_{\min} \quad C_{2,T_2} = e^{\mu_{\max}} \Big/ \left(\frac{1}{C_{1,T_1}} + \frac{1}{C_{\max}} \left(e^{\mu_{\max}} - 1 \right) \right) \\
&T_2 \le T_{\min} \quad C_{2,T_2} = C_{1,T_1} \\
&\qquad \vdots \\
&T_n > T_{\min} \quad C_{n,T_n} = e^{\mu_{\max}} \Big/ \left(\frac{1}{C_{n-1,T_{n-1}}} + \frac{1}{C_{\max}} \left(e^{\mu_{\max}} - 1 \right) \right) \\
&T_n \le T_{\min} \quad C_{n,T_n} = C_{n-1,T_{n-1}}
\end{aligned} \tag{21}$$

6.10.3 Assumptions

It is assumed that there is no lag-phase for SE. A lag phase is usually observed after sudden changes in environmental circumstances, requiring physiological processes in bacterial cells to adapt before growth continues. In case of climate change, the circumstances change gradually and bacteria can adapt their physiologically processes similarly.

6.10.4 How to enter data in the tool

S. Enteritidis grows rapidly in the egg yolk (Bradshaw et al., 1990; Gast and Holt, 2000), but yolks of naturally infected eggs are not frequently contaminated with *S. Enteritidis*. Generally, salmonellae are found in the albumin of naturally infected eggs (Humphrey et al., 1989a; Mawer et al., 1989). The probability of an egg being contaminated has a default value of 0.005 (EFSA, 2010), but can be given another value manually by pressing the 'set'-button. Initial concentrations in a contaminated egg can be selected (2, 25, 250 and 2500 CFU/g), from which the probability of growth is derived (Cogan et al. ,2001), or can be set to an intermediate value. Probabilities of growth in an egg, the occurrence of low and high contaminated eggs are shown. The days that eggs are exposed to ambient air temperature (for example on a market place) can be chosen (0 – 7 days). On the right half of the screen the concentration

of SE in eggs in which SE has grown (high contamination) after a number days exposure to ambient air is shown for each day of the year under current (blue) and future (red) climate conditions.

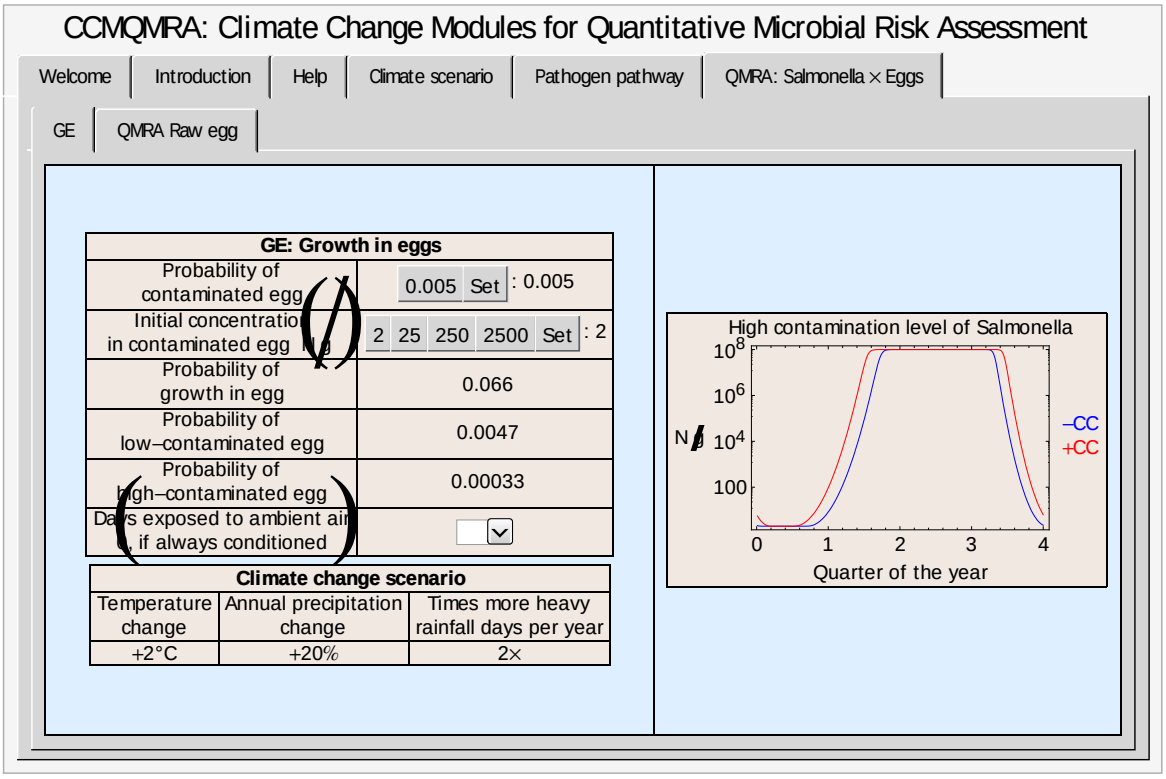

Figure 13 GE: Screen image of Growth in Eggs

6.11 Module PPF: Prevalence in Poultry Flocks

6.11.1 Pathogens

Campylobacter

6.11.2 Mathematical description

A relation between temperature and *Campylobacter* incidence in broiler flocks in Denmark was described by Patrick et al. (Patrick et al., 2004). The fractions of *Campylobacter* positive broiler flocks at temperatures of 0°C, 5°C, 10°C, 15°C and 20°C were 0.2, 0.25, 0.4, 0.6 and 0.8, respectively. A logistic model, fit for limiting values of a response variable (i.e., campylobacter prevalence) between '0' and '1', was used to describe these data. The estimated value for the intercept was -1.30, the estimate for the regression parameter was 0.007, resulting in the following equation for estimating the prevalence P and the temperature T :

$$P(T) = \frac{e^{-1.30+0.007T^2}}{1+e^{-1.30+0.007T^2}} \qquad (22)$$

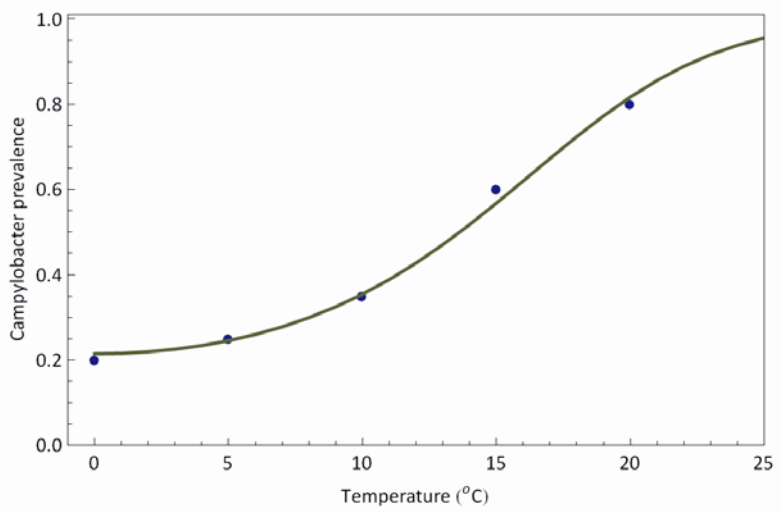

Figure 14 Relation between temperature and *Campylobacter* prevalence among broiler flocks

Parameter P represents the number of *Campylobacter* positive broiler flocks that enter the slaughter house. During slaughter, uncontaminated flocks can become cross-contaminated by a contaminated flock (Evers, 2004). This cross-contamination during slaughter will result in a larger number of contaminated chicken fillets in retail stores and should be included in a risk assessment. Depending on the type of slaughter, either random or logistic, the number of cross-contaminated flocks will differ. During random slaughter, (*i.e.*, broiler flocks are slaughtered in random order on a day) the number of cross contaminated flocks will depend on the slaughter order. With logistic slaughter, all flocks are tested for *Campylobacter* presence prior to slaughter. Flocks that test *Campylobacter* negative are slaughtered before those that tested *Campylobacter* positive. When a diagnostic test with 100% sensitivity is used, then there will be no cross-contamination. However, such tests do not exist and therefore some cross contamination will still occur. The magnitude of cross-contamination depends on the sensitivity of the entire testing protocol, because false-negative test results may cause campylobacter-positive flocks to be slaughtered early in the cascade, enabling cross-contamination between flocks despite logistic slaughtering.

Prevalence in flocks after slaughter is calculated by using the analytical formulas for the maxium effect of logistic slaughter as described in detail by Evers (Evers, 2004).

6.11.3 Assumptions

It is assumed that every broiler that is raised in a *Campylobacter*-positive flock produces contaminated chicken fillets and every broiler that is raised in a *Campylobacter*-negative broiler flock produces *Campylobacter*-free chicken fillets. Furthermore, it is assumed that all chicken fillets from a contaminated flock are contaminated, which may be an overestimation.

It is assumed that *Campylobacter* does not grow on chicken fillet. This assumption is valid considering that *Campylobacter* cannot grow below temperatures of 30°C (Jacobs-Reitsma et al., 2008). And lastly, it is assumed that each broiler flock is of the same size. This means that the *Campylobacter* status is considered to be independent of flock size. This aspect may not be correct, because some studies report a correlation between farm size and *Campylobacter*-positivity (e.g. Bouwknegt et al., 2004). Other studies, however, did not find such a relation (van Asselt et al., 2008). Because of this ambiguity, a relation between flock size and *Campylobacter* status has not been included in the tool.

6.11.4 How to enter data in the tool

The PPF module shows the function of prevalence of *Campylobacter* in poultry flocks as a function of temperature, before flocks are slaughtered. On the right half of the screen this prevalence in plotted for each day in the year under current (blue, dotted) and future (red, dotted) climate conditions. In the slaughter house, slaughter is either random or logistic. The number of slaughtered flocks per day can be selected (1 – 10). The number of flocks following a contaminated flock that get cross-contaminated (1 – (number of slaughtered flocks -1) can be selected. In the case of logistic slaughter, the sensitivity of the research protocol to detect *Campylobacter* contamination can be entered (0 – 1).
On the right half of the screen the prevalence after slaughter for each day of the year under current (blue, solid) and future conditions (red, solid) is shown.

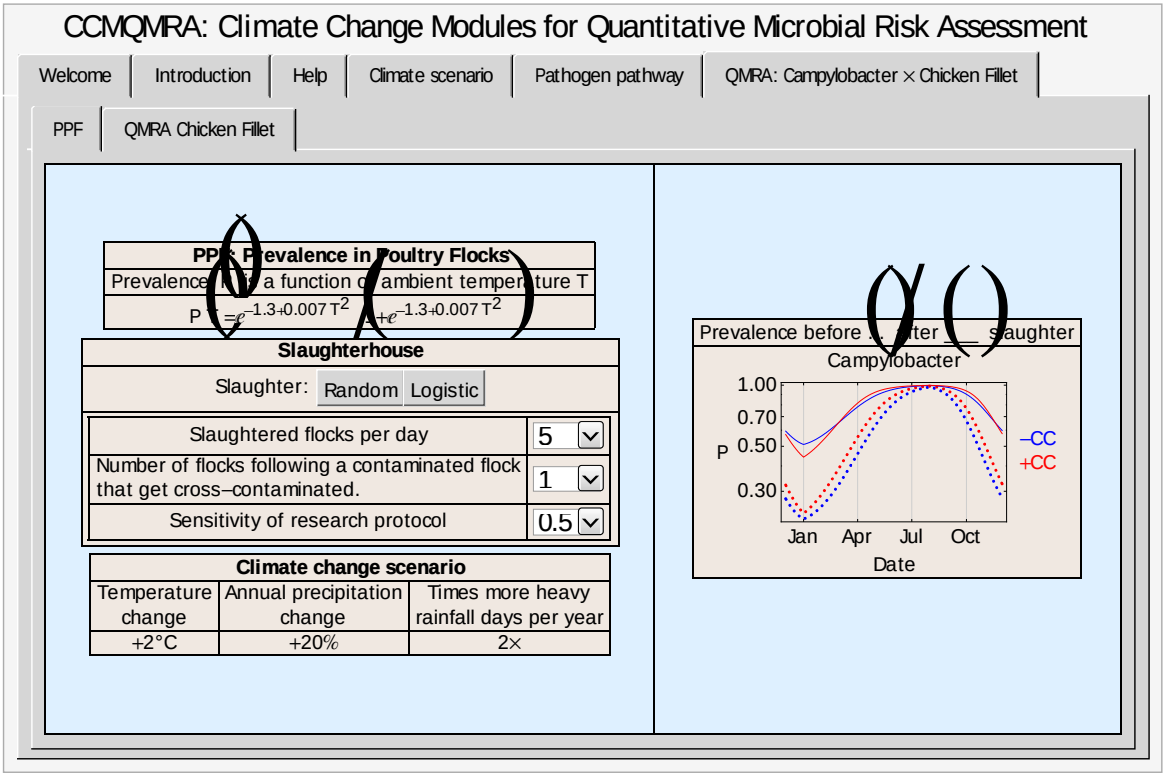

Figure 15 PPF: Screen image of Prevalence in Poultry Flocks

6.12 Module TDW: Drinking water treatment

6.12.1 Mathematical description

This module is part of the QMRA Pathogen × Drinking Water screen.

Surface water is treated by a number of consecutive treatments to remove pathogens. Treatment steps entail filtration and disinfection processes. Efficiency of drinking water treatment steps may vary widely and are highly location-specific. An overview of indicative values for treatment efficiency is given in the WHO drinking water quality guidelines (WHO, 2008), In addition, numerous publications have appeared on pathogen removal by drinking water treatment. In the tool, drinking water treatment is entered as $\log_{10} Z$ and is assumed to follow a normal distribution. Z is the fraction of pathogens that is able to pass a treatment.

6.12.2 Assumptions

Drinking water treatment is assumed to be normally distributed on a log scale.

6.12.3 How to enter data in the tool

Data for TDW are entered on the QMRA Drinking Water screen. The user enters the mean $\log_{10}$ value of the total treatment efficiency for drinking water together with the standard deviation of this mean. The total treatment includes the sum of each of the individual treatment steps on a log scale. For instance, Treatment step A with 2 $\log_{10}$ units reduction in viral load and Treatment step B with 1.5 $\log_{10}$ units decrease in viral load mounts to a total treatment efficiency of 3.5 $\log_{10}$ units. These entries are used to generate a normal distribution for the treatment efficiency on a $\log_{10}$-scale, from which values are randomly drawn during Monte Carlo simulation. From the surface water concentrations at the point of intake for drinking water production and the fraction Z of pathogens that are able to pass treatment the pathogen concentrations in drinking water are calculated.

6.13 Module VDW: Volume of unboiled drinking water consumption per person per day

6.13.1 Mathematical description

This module is part of the QMRA Pathogen × Drinking Water screen.

The model uses either a distribution of unboiled water consumption per person per day based on Dutch data (Teunis et al., 1997) with an average of about 0.25 liter per person per day, or based on USEPA data (USEPA, 2006) with an average of 1 liter per person per day, or a point estimate of 2 liter per person per day (WHO, 2008). The NL-data are a lognormal distribution with parameters -1.85779 and 1.07487 and the USEPA data are a lognormal distribution with parameters -0.03598 and 0.77218.

From the pathogen concentrations in drinking water and the drinking water consumption the dose D (exposure) is calculated, which is the numbers of pathogen that are ingested per person per day.

6.13.2 How to enter data in the tool

In the QMRA Drinking Water screen, one the three options NL, USEPA and WHO can be selected.

6.14 Module VBW: Volume of swallowed Bathing Water

6.14.1 Mathematical description

This module is part of the QMRA Pathogen × Bathing Water screen.

The module is restricted to bathing in fresh water. The module uses the data from Schets et al (in preparation). In this study, the volume of swallowed water per bathing event was estimated from inquiry data. A distinction could be made between men, women and children. On average men swallowed 27 ml of water, women 18 ml and children 37 ml. Because of the applied dose response relation, the exposure data of the men are used. It should be noted that children swallow more water and swim more often than men. And therefore, exposure of children is higher. However, the dose response relation is not speeific for children. In the risk assessment, a distribution for the volume of swallowed bathing water by men is sampled by Monte Carlo simulation from a Gamma distribution with parameters $\alpha = 0.45$ and $\beta = 60$.

From the pathogen concentrations in bathing water and the volume of swallowed bathing water, the dose D (exposure) is calculated, which is the numbers of pathogen that are ingested per person per bathing event.

6.14.2 How to enter data in the tool

The minimum swimming temperature that a person goes swimming can be entered.

6.15 Module COY: Consumption of Oysters.

6.15.1 Mathematical description

This module is part of the QMRA Pathogen × Oyster screen.

From the pathogen concentrations in oysters and the oyster consumption the dose D (exposure) is calculated, which is the numbers of pathogen that are ingested per person per meal of oysters.

6.15.2 How to enter data in the tool

A value of 20, 50, 100 or 200 gram can be entered on the QMRA Pathogen×Oyster screen.

6.16 Module CE: Consumption raw/undercooked Egg.

6.16.1 Mathematical description

This module is part of the QMRA Pathogen × Egg screen.

From the pathogen concentrations in egg(product) and the consumption of undercooked/raw egg(product) the dose D (exposure) is calculated, which is the numbers of pathogen that are ingested per person per meal.

6.16.2 How to enter data in the tool

One egg (52 g), 100 gram egg product or 200 gram egg product can be entered on the QMRA Pathogen×Egg screen.

6.17 Module CCF: Consumption raw/undercooked Chicken Fillet.

6.17.1 Mathematical description

This module is part of the QMRA Pathogen × Chicken Fillet screen.

From the pathogen concentrations in chicken fillet and the consumption of undercooked/raw chicken fillet, the dose D (exposure) is calculated, which is the numbers of pathogen that are ingested per person per meal.

6.17.2 How to enter data in the tool

A concentration of *Campylobacter* in chicken fillet of 1 (L), 10 (M), 100 (High), or any value (Set) can be entered and for consumption, a value of 20, 50, 100 or 200 gram can be entered on the QMRA Pathogen×Oyster screen.

6.18 Module DR: dose-response model

6.18.1 Mathematical description

The beta-Poisson model is used. This model accounts for variability in dose response. The formula for calculating the risk of infection for a specific dose D is calculated as follows (Teunis et al., 2008):

$$P_{\text{inf}} = 1 - {}_1F_1(\alpha, \alpha + \beta; -D) \tag{23}$$

where α and β are infectivity parameters that are pathogen specific (see Table 6) and ${}_1F_1$ is the confluent hypergeometric function. In the case of *Salmonella*, $\beta >> \alpha$, implying very slow calculation of infection risks may occur. To avoid that, for this pathogen, infection risk is approximated as follows (Teunis and Havelaar, 2000):

$$P_{\text{inf}} = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha} \tag{24}$$

6.18.2 Assumptions

For the exponential dose response model, it is assumed that each pathogen has the same probability of infection and that each host is equally susceptible to infection. In case of the beta-Poisson dose response model, it is assumed that hosts are heterogeneous in susceptibility.

6.18.3 How to enter data in the tool

No data need to be entered for this module. The tool selects the correct parameters automatically.

Table 14 Dose response parameters

Pathogen	α	β	Reference
Norovirus	0.04	0.055	Teunis et al. (2008)
Campylobacter	0.038	0.022	Teunis et al. (1995)
Cryptosporidium	0.106	0.295	Teunis et al. (1996)
Vibrio	0.136	0.149	Teunis et al. (1996)
Salmonella	0.4047	5587	WHO/FAO (2002)

6.19 QMRA Drinking water

6.19.1 Pathogens

Norovirus, *Campylobacter*, *Cryptosporidium*

6.19.2 Mathematical description

The pathogen concentrations in the surface water at the point of intake for drinking water production are the starting point of this part of the risk assessment. This part encompasses the modules TDW (Treatment of Drinking Water), VDW (Volume of unboiled Drinking Water per person per day), DR (Dose Response) and Risk of Infection. These modules are combined on the same screen.

In QMRA for Drinking Water, first infection risks are calculated per person and per day using equation (23). In the case of drinking water, the infection risk per person per year is of interest. This is achieved by sampling from the Monte Carlo sample of infection probabilities from a subset of 365 probabilities p_i and calculating:

$$P_{inf,year} = 1 - \prod_{i=1}^{365} (1 - p_i) \qquad (25)$$

The relative risk due to climate change is calculated by dividing the infection risk after climate change by the estimated infection risk before climate change. This is done for each of the iterations in the Monte Carlo sampling, yielding a range of relative risks depicted in a histogram. The mean relative risk and 95% interval are presented as output of the tool.

6.19.3 Assumptions

No other assumptions are made than those described in the modules CSO, RO, TDW, VDW, DR and Risk of Infection.

6.19.4 How to enter data in the tool

Entering data for TDW and VDW is already described in previous sections. The screen shows a dose response curve of the selected pathogen to inform the user about its infectivity. For example, the ID_{50} value, which is the number of pathogens that have a probability of 50% to cause an infection, can be read from this curve.
A Target Value can be set. In the Netherlands, this equals 10^{-4} (one per ten thousand persons per year) by law (Anonymous, 2001). Otherwise, the user may determine what risk level is acceptable or not. An infection risk about the Target Value appears red and otherwise green in the box-whisker plots shown on the right half of the screen for current and future climate conditions.

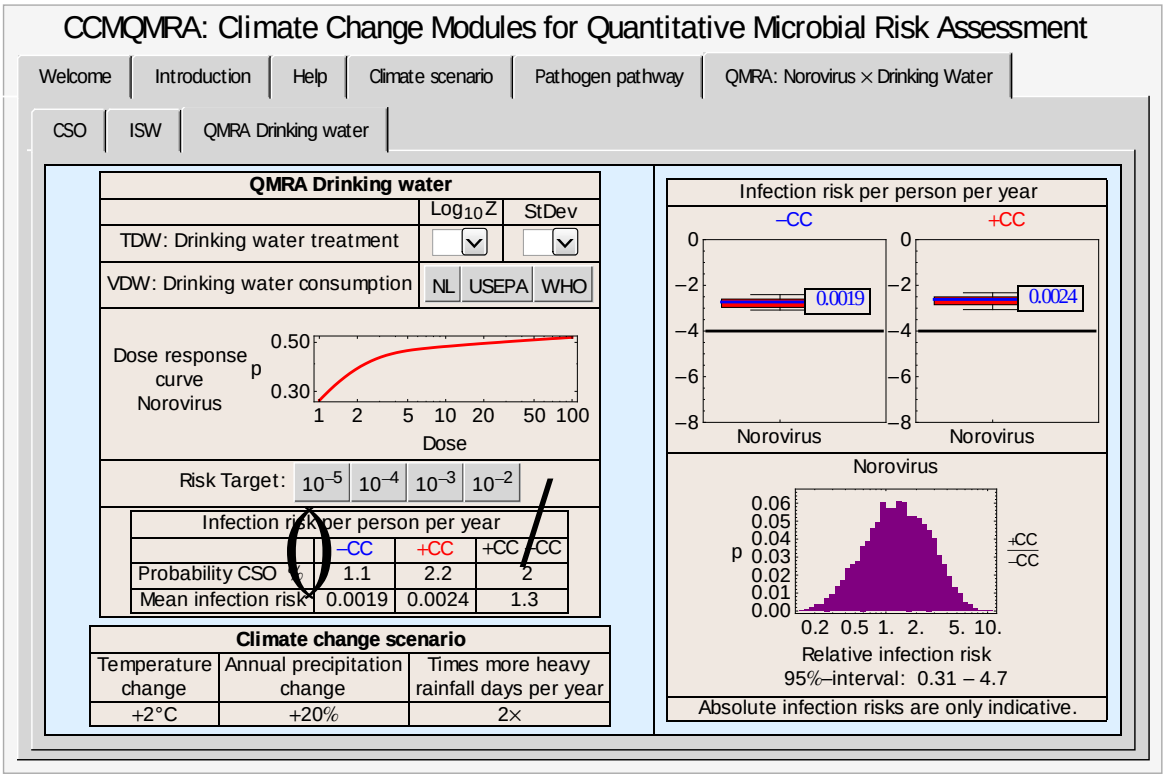

Figure 16 Screen image of QMRA Drinking Water

6.19.5 How to interpret the outcome of the tool

Note the warning that absolute infection risks are only indicative, because of simplifications, point estimates, lack of data. Moreover, generally variability is wide, each new risk calculation may be different, because each is a realisation from Monte Carlo sampling.

A relative risk of “1” means that the infection risk for the current situation and the situation after climate change are similar. A relative risk <1 means that the infection risk after climate change is lower than the risk in the current situation, a relative risk >1 means that the infection risk after climate change is higher than the risk in the current situation.

The 95% interval gives the range of relative risks that were calculated and shows the variation of the relative risk due to the variation reported for the parameters. If the 95% interval excludes “1”, then it is most likely that–based on the entered data–the infection risk due to climate change will increase (for values >1) or decrease (for values <1).

6.20 QMRA Bathing Water

6.20.1 Pathogens

Norovirus, *Campylobacter*, *Cryptosporidium, Vibrio*

6.20.2 Mathematical description

The pathogen concentrations in the surface water at the bathing water area are the starting point of this part of the risk assessment. This part encompasses the modules VBW (Volume of swallowed Brinking Water per person per bathing event), DR (Dose Response) and Risk of Infection. These modules are combined on the same screen. In QMRA for Bathing Water, infection risks are calculated per person and per bathing event using equation (23).

6.20.3 Assumptions

No other assumptions are made than those described in the modules CSO, RO, VBW, DR and Risk of Infection.

6.20.4 How to enter data in the tool

Entering data for VBW is already described in a previous section. The screen lay-out is the same as described for drinking water in section 6.19.4.

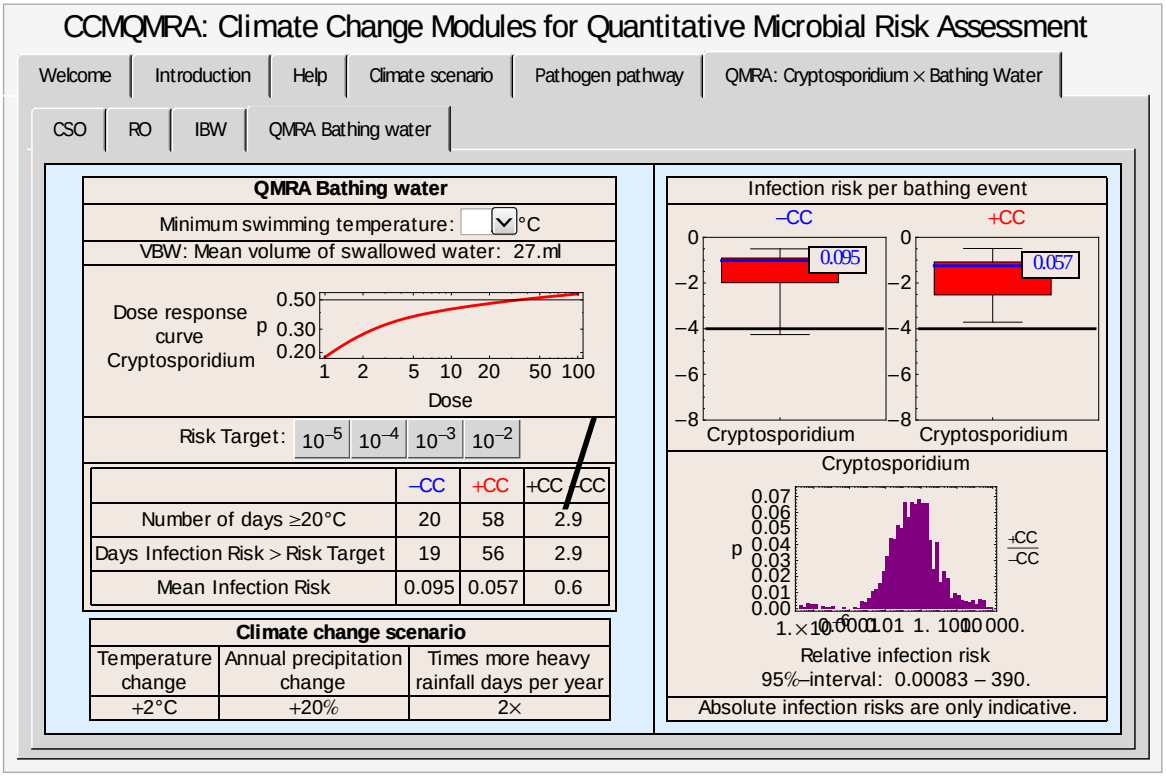

Figure 17 Screen image of QMRA Bathing Water

6.20.5 How to interpret the outcome of the tool

See section 6.19.5. In addition to estimates of absolute and relative infection risks, the number of days above the minimum swimming temperature are of relevance too, because it relates to the opportunities to go swimming. In the case of a temperature increase between current and future climate conditions, there is an increased opportunity for swimming.

6.21 QMRA Oysters

6.21.1 Pathogens

Norovirus, *Campylobacter*, *Cryptosporidium*, *Vibrio*

6.21.2 Mathematical description

The pathogen concentrations in raw oysters are the starting point of this part of the risk assessment. This part encompasses the modules COY (Consumption of raw oysters), DR (Dose Response) and Risk of Infection. These modules are combined on the same screen. In QMRA for Oysters, infection risks are calculated per person and per consumption of oysters using equation (23).

6.21.3 Assumptions

The concentration of pathogens for each of the consumed oyster is considered constant. In practice, this concentration will vary between oysters.

6.21.4 How to enter data in the tool

Entering data for COY is already described in a previous section. The screen lay-out is the same as described for drinking water in section 6.19.4.

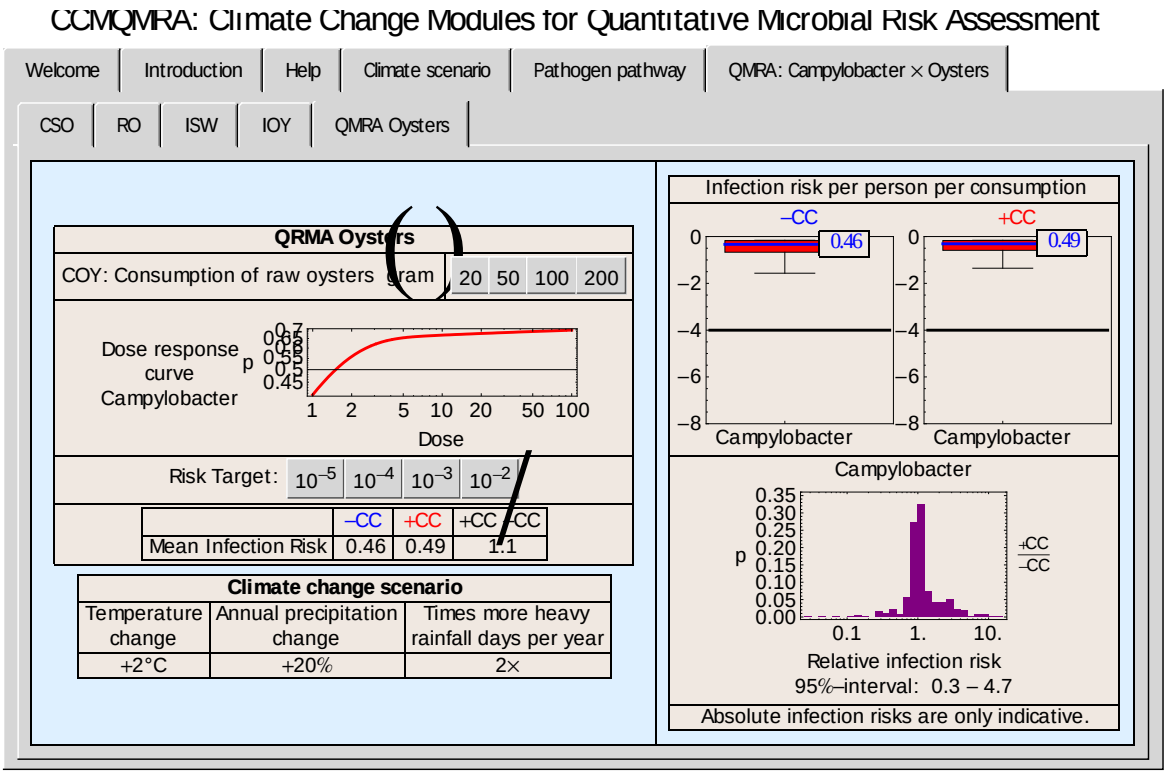

Figure 18 Screen image of QMRA Oysters

6.21.5 How to interpret the outcome of the tool

See section 6.19.5.

6.22 QMRA Eggs

6.22.1 Pathogens

Salmonella

6.22.2 Mathematical description

The pathogen concentrations in egg (low and high level of contamination) are the starting point of this part of the risk assessment. This part encompasses the modules CE (Consumption of raw/undercooked egg(product)), DR (Dose Response) and Risk of Infection. These modules are combined on the same

screen. In QMRA for Eggs, infection risks are calculated per person and per consumption of an egg or eggproduct using equation (23). In the case of single egg consumption, the risk of infection from eating an egg with the dose as the number of *Salmonella* in that egg multiplied by the probability of having a contaminated egg. In the case of eggproduct, which is part of a mixture of a large numbers eggs, the dose is the number of *Salmonella* in an infected egg is multiplied by the probability of a contaminated egg. In the latter case, the probability of a contaminated egg is effectuated as a dilution factor.

6.22.3 Assumptions

No other assumptions are made than those described for the modules GE, DR and Risk of Infection.

6.22.4 How to enter data in the tool

Entering data for CE is already described in a previous section. The screen lay-out is the same as described for drinking water in section 6.19.4.

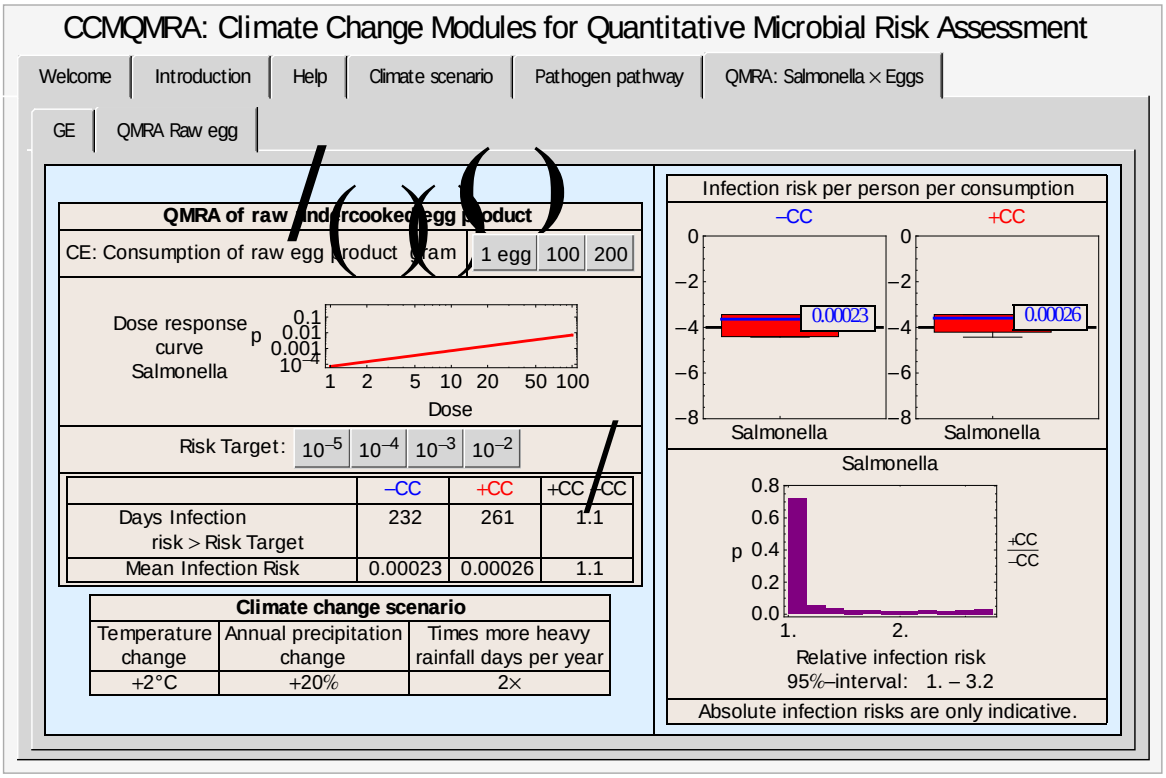

Figure 19 Screen image of QMRA eggs

6.22.5 How to interpret the outcome of the tool

See section 6.19.5.

6.23 QMRA Chicken Fillet

6.23.1 Pathogens

Campylobacter

6.23.2 Mathematical description

The pathogen concentrations in chicken fillet are the starting point of this part of the risk assessment. This part encompasses the modules CCF (Consumption of raw/undercooked chicken fillet), DR (Dose Response) and Risk of Infection. These modules are combined on the same screen. In QMRA for Chicken Fillet, infection risks are calculated per person and per consumption of an amount of chicken fillet using equation (23).

6.23.3 Assumptions

No other assumptions are made than those described in the modules PPF, CCF, DR and Risk of Infection.

6.23.4 How to enter data in the tool

Entering data for CCF is already described in a previous section. The screen lay-out is the same as described for drinking water in section 6.19.4.

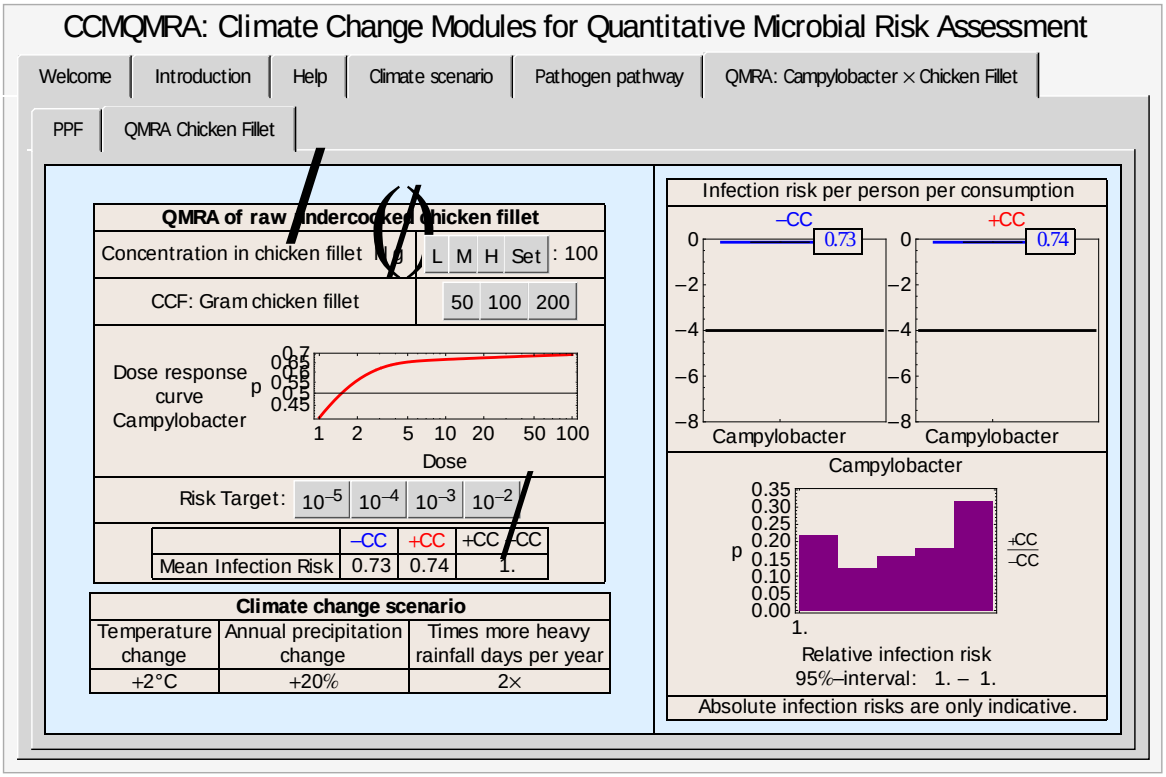

Figure 20 Screen image of QMRA Chicken Fillet

6.23.5 How to interpret the outcome of the tool

See section 6.19.5.

7 Pilot testing of the tool

7.1 Introduction

A pilot version of the tool was sent to eight people with different expertise to test the tool on several aspects. These aspects include:

- the relevance of selected modules to assess the impact of climate change on FWD
- the relevance of selected pathways to assess the impact of climate change on FWD
- the mathematical solidity of the tool
- the user-friendliness of the tool
- the practical use of the tool (i.e., can all local parameters that are considered relevant for climate change by users be entered in the tool)

The different expertises that were represented by the test consortium are detailed in paragraph 7.2 of the current report.

7.2 Pilot testing scheme

The first pilot testing (version 1.0) was conducted by Andrea Rechenburg and Christoph Höser of UKB/UNI-Bonn, who are the experts in incidences of infection diseases in relation to climate changes (responsible for Lot1 of the current project). The comments and suggestions were received by telephone on June 6th, 2010. The revised tool (version 1.1) was subsequently sent on June 11, 2010 to the contractors (Jan Semenza and Jonathan Suk, ECDC) for further testing Comments and suggestions were exchanged during two phone meetings on July 29th and 30th, 2010. Furthermore, version 1.1 of the tool was discussed at RIVM with Peter Teunis, a renowned expert in quantitative microbial risk assessment. On August 16, the tool (version 1.2) was sent to Elsa Casimiro from INFOTOX, Portugal. Dr. Casimiro served as an expert on climate change and provided useful suggestions on the practical use of the tool. Comments were received by email on August 18th, 2010. Furthermore, two colleagues at RIVM with limited experience in either modelling or climate change were consulted for testing the practical use of the tool and the clarity of the presentation of results.

7.3 Comments and suggestions received

1. The tool is seen as a tool for experts, being climatologists or microbiologists, who are therefore only familiar with part of the field the tool covers. It was suggested to include a TAB with an introduction of the tool, its purpose and background information. This has now been done.
2. It was suggested to include preset climate change scenarios. This has been done. One may choose from fours scenarios, or set a scenario.
3. A multiplication sign (for the increase factor of heavy rainfall) has been included.
4. The data scales are not clear. This issue is recognized, the date scale is changed to quarters of the year.

5. The tool contains many so-called “tool tips”: if the mouse goes over words, help information appears. Under the word "Modules" under the TAB "Case", it is now shown what all the checkboxes mean.
6. The impression existed that raw wastewater concentrations are dependent on climate change. This is not the case, it depends on a rain fall event. The choice of red and bleu colors was misleading. This been changed. Also, in every plot blue and red are only used to designate -CC and +CC.
7. The tool has been changed regarding the rain peaks: peaks can now be set randomly for each quarter of the year, so one can have them uniformly distributed over the whole year, or some quarter may have more peaks than other quarters.
8. The duration of rainfall is set at the climate scenario page. Frequency of CSO and RO are assumed to be the same due to the rain peaks.
9. The tables and figures in QMRA-BW are complicated and overwhelming. Rearrangement of columns and rows was suggested. This has been done.
10. The meaning of risk target has been added to the TAB "Help", but will also be included as tool tip.
11. The Monte Carlo buttons are confusing. This is recognized and a solution was included in the updated version.
12. One needs to go step-by-step through the tool for updating settings. Solution: The next step is now only available if calculations of the previous step are done. Availability is clear from the presence/absence of the TAB for the next step.
13. Drought should be included in the model as climate change factor. Drought could have an impact on the flow rate of the river and thereby on time for inactivation or growth, and also on dilution of discharged pathogens. It may also affect runoff of pathogens from agricultural land.
14. The target group and the objectives of the tool are not clear. This is now made clear on the welcome-screen. The scope of the tool needs to be made clear.
15. A disclaimer was suggested to make clear that application of the tool and interpretation of the outcomes is the responsibility of the end-user, not of the developers.
    Such a disclaimer will be included as well as an explanation with the limitations of the tool.
16. Present relative risks as a distribution of the ratio of the risks estimates before and after climate change. This has been done, including 95%-interval.
17. It will be useful to compare the outcomes of the tool for a number of the most important case studies with the results of more detailed case studies. This is recognized as an important step and will be done.
18. More explanation is needed about the implementation of models, the models that were selected. This is done in the report and under the help tab.
19. By means of a sensitivity analysis it is possible to decide if a parameter needs to be included as a random factor (with a distribution) or only as a point estimate, if the parameter has little effect on the outcome. This is done.
20. Testing against a target value is only useful if the risk estimate is a distribution.
21. Dose response relation for *Salmonella*: This is a population estimate from a number of outbreaks with variation between the outbreaks. It is better to use a mixed model that accounts for differences in infectivity/pathogenicity between strains from different outbreaks. Soon a paper of Strachan et al. (in press) is to appear, where dose response is base on more extensive data. It requires a Monte Carlo sample of parameters, in stead of two fixed parameters.

22. Dose-response relation for *Vibrio*: Two exist, one for an effective pH barrier in the stomach, and one for persons with no such pH barrier. Note provisionally that there is uncertainty about the use of this dose response relation.
23. The Mathematica 7 Player seems to be a problem on computers working on the 64-bit version of Windows Vista. The software may not install correctly. Upon inquiry at Wolfram Inc. Mathematica Player was stated to work normally on a 64-bit machine.
24. It might be a good idea to add a flow diagram with how this tool works in a manual.
25. To be location specific, the tool needs to be more suitable for entering current and future weather in southern Europe. Also an explanation for using weeks was desired.
26. Current climate: the highest possible temperature for T_{min} in air is 15^{o}C. In Southern Europe T_{min} is often above this value. It is suggested to increase T_{min} possible values to about 25^{o}C.
27. The same applies to the water T_{min} values.
28. Likewise, the air and water T_{max} values need to be increased. In summer, some countries, especially those from Southern Europe, may be hot, but not as hot as Spain and Greece, because Portuguese weather is influenced by the Atlantic Ocean.
29. The custom option for change in temperature "*delta*" range should be increased. Furthermore, it would be good to indicate what you are referring to: the mean temperature, T_{max} or T_{min}. (note: new comers to the climate change impact assessments might not be able to understand the large differences between the IPPC type increase temp by 2^{o}C (which is meant as a global average), to a local study that needs to be done at the local level, where the change can be much bigger. This needs some explanation.
30. It would be valuable to include a worked out example of a case study and explain how and why particular parameters were entered in the tool.

7.4 Follow-up

Each of the comments has been considered carefully. The tool has been improved by adoption of all of the suggestions in the tool. Furthermore, the comments and suggestions about explanation of the tool have been included in the glossary of the tool, on the help pages, and in the current report.
The problems that arose when installing Mathematica player on computers running Windows Vista 64-Bit has been forwarded to Wolfram Inc. for suggestions. On August 31st, no response was obtained.

8 Conclusions and recommendations

This first version of the CCMQMRA tool includes a total of thirteen QMRA combinations, each existing of a set of consecutively linked modules selected from the 22 modules created. The tool is highly versatile: the thirteen QMRA combinations can be run under various location-specific current climate conditions throughout Europe, projected climate conditions and specific data depending on the selected modules. Outcomes of the tool are estimates of infection risks for current and future climate conditions and relative infection risks. The tool gives an estimate of the direction (increase or decrease) and magnitude of the relative infection risks for the selected pathogens due to climate change. The estimates of the infection risks should be regarded as indicative estimates in the order of magnitude, because of the use of point estimates (single average values) and simplification of models due to data deficiencies and assumptions.

For completing a specific QMRA, insufficient data may be available. In such cases, default values may be selected for the QMRA, but the representativeness of the default values for the specific situation needs to be assessed and documented. The estimated relative risks are as accurate as the entered data allow. Alternatively, in case of insufficient data, the tool can be used to identify the data gaps and to direct future data collection. This can be done by selecting the desired pathogen-pathway combination and writing down the required entry fields for each of the modules.

The CCMQMRA tool is exceedingly suitable for answering ‘what-if’ questions for specific pathogen-pathway combinations. For instance, the required improvement in treatment efficiency of drinking water production to not exceed a certain threshold infection risk due to the consumption of unboiled drinking water can be assessed. Similarly, the effect of travel distance from a source of contamination to, for example, a bathing area can be analysed. Another example of a what-if scenario involves the percentage of *Salmonella* contaminated eggs. Currently, data are lacking to relate the changing climate to the prevalence of *Salmonella* contaminated eggs. Nevertheless, if one is interested in the increase in infection risks when the prevalence of *Salmonella* contaminated eggs would increase from 5% to 10%, then the respective QMRA could be completed twice using the same climate change scenarios, once for the 5%-prevalence situation and once for the 10%-prevalence situation. The relative infection risk is then calculated by dividing the estimate for the 10%-prevalence situation after climate change (obtained from the Box-Whisker plot) by that estimate for the 5%-prevalence situation.
In general: a variety of `what-if`` scenarios can be investigated as a basis for defining adaptation strategies or preventive measures by comparing relative risks from varying location-specific parameter values.

The current tool can be improved by including more and better estimates of parameter values for the fate and behaviour of the pathogens once these become available. More difficult is the inclusion of additional growth or inactivation kinetics models. Such updates require specific knowledge on the programming in Mathematica. For instance, the current growth model for *Vibrio* is a simple model, and may be replaced by a growth model that accounts for temperature, pH, salinity, type of *Vibrio*, and occurrence of *Vibrio* in water once suitable such data become available. Nevertheless, the tool is created such that future knowledge can be included in future versions of the tool.

The current tool is designed for use on a local scale, *i.e.* for a community or small region and cannot estimate the relative infection risks for the whole of Europe instantaneously. To obtain an indication of the estimates for Europe, location specific data (climatic and non-climatic) could be obtained for smaller regions in Europe (e.g., from municipal health services). These data could subsequently be entered in the QMRA for selected pathogen-pathway combinations. The resulting array of relative risks

could be used to generate maps of Europe showing by colour variation the differences in estimated relative infection risks. Such maps will indicate the areas that are estimated to be affected most by climate change in terms of food- and water-borne infectious diseases. Therefore, such maps would yield valuable information for public health authorities as indicator to include food- and water-borne diseases in their adaptation strategies based on expertise in QMRA modelling.

The tool estimates the relative infection risk for each of the pathogens. For public health authorities, it may also be valuable to add to the tool estimates of the disease burden caused by the pathogen-pathway combinations. For instance, by including estimates of *disability adjusted life years* (DALYs) caused or saved by climate change, prioritization in adaptation strategies for food- and water-borne pathogens could be supported. Furthermore, by adding DALY estimates, it will also be possible to estimate not only a pathogen-specific reduction or increase in relative risk, but to estimate a combined effect for the presence of a set of pathogens. For instance, the presence of norovirus, *Vibrio* and hepatitis A virus in oysters may change differently due to specific climate changes. In case the burden for e.g. *Vibrio* increases, and the burden for the other two pathogen decreases, then the total disease burden due to specific climate changes may decrease (or increase). Such information will support public health authorities to prioritize for pathogen pathways rather than pathogens, which is relevant for developing adaptation strategies.

9 References

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