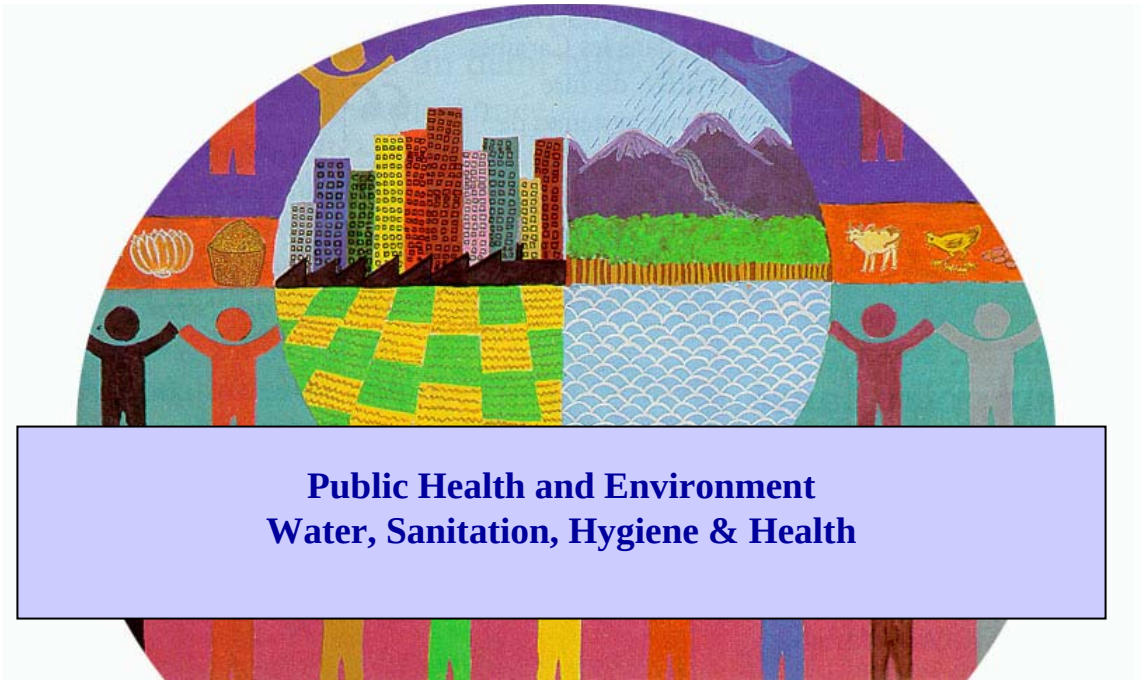
World Health Organization

Risk Assessment of Cryptosporidium in Drinking Water



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PREFACE

Background

In the process of revision of the WHO Guidelines for Drinking Water Quality, the need for a fundamental change in the guidelines for microbial safety has been identified. The former Guidelines were focussed on end-product monitoring for *E. coli*. This system is reactive; the warning signal is received at the time that the consumer's health is already at risk. Outbreaks of disease through drinking water that meets this Guideline have been reported. This also indicates that meeting the Guideline is not always a safeguard against transmission of illness through that same drinking water. Developments in microbial risk assessment and in a risk management framework in the food industry have indicated that a preventive, risk based approach can provide the necessary expansion of the current approach to protect the consumer against health effects from drinking water.

In subsequent meetings in Medmenham (1994), Stockholm (1999), Berlin (2000) and Adelaide (2001), the microbiology working group of the revision of the WHO guidelines has been progressing towards the complementation of the current microbiological guidelines with the requirement for a Water Safety Plan. Such a plan is a systematic inventory of the hazards, an evaluation of the significance of these hazards and of the efficacy of control measures taken. This changes the focus of attention to verification that the safeguards in the water supply chain (catchment and source protection, treatment processes, distribution system integrity) are in place and effective.

In this new approach to the new Guidelines, the need for background documents that illustrate the approach and discuss the available scientific information was identified. This document on *Cryptosporidium* is the first in a series of microbiological Environmental Health Criteria (EHC) that will serve as background documents.

Cryptosporidium is selected as the target for this risk assessment. Its ubiquitous occurrence in the environment, its persistence, and resistance to chemical disinfection has made this protozoan parasite to one of the critical pathogens for the drinking water industry. Numerous drinking water outbreaks have been reported since its first recognition as a waterborne agent in 1984. Most of these were attributed to insufficiencies or failures in water treatment and distribution, but more importantly many occurred in systems that were regarded as safe and complied with the microbiological standards. As a consequence, research has focussed on

this pathogen over the last decades and has provided a wealth of information, on sources, occurrence and behaviour of *Cryptosporidium* in water, on removal and inactivation by water treatment processes and on its pathogenicity. Risk assessment requires this type of knowledge.

This document follows the basic steps of the microbial risk assessment framework: Hazard Identification, Problem Definition, Exposure assessment, Effect assessment and risk characterisation. The chapters follow the subsequent steps and show both the information that is needed to complete the step and the information that is available about *Cryptosporidium*. The document aims to illustrate what it means to implement the risk assessment framework in the drinking water supply, both in terms of the information that is required and in terms of the information that it provides to aid risk management. It discusses the information on *Cryptosporidium* that is available to incorporate in this process and also highlights the information that is still lacking

Target audience and purpose

The target audience of this document are persons responsible for:

- setting standards for drinking-water,
- evaluating adequacy of drinking-water quality or water treatment, and /or
- system design, implementation, and supervision in controlling infectious disease.

They can use this document as guidance for a quantitative assessment of the health risk of *Cryptosporidium* through a drinking water supply. For systems that have no specific data on *Cryptosporidium,* we have deduced default source water concentrations for different types of source waters. Similarly, we have deduced default log-credits for surface water treatment processes. We have included several worked-out case studies to illustrate the approach, the information that is needed and the result it provides and how this can be used in risk management. In these case studies, we have tried to show the spectrum from systems where very little specific information about *Cryptosporidium* is available to systems that have site-specific information about *Cryptosporidium* in their source water and removal by water treatment. We feel that this illustrates the power and versatility of the new risk assessment approach. The approach can be effectively applied in all cases, from a simple screening study to sophisticated collection and statistical evaluation of comprehensive data-sets covering all steps of the risk assessment.

1

Cryptosporidium as reference pathogen

1.1 FRAMEWORK FOR SAFE DRINKING-WATER

In the 3rd edition of the Guidelines for Drinking Water Quality, the World Health Organisation [WHO, 2004] has introduced the preventive management *Framework for Safe Drinking-water* that comprise five key components (Figure 1):

- Health based targets based on critical evaluation of health concerns;
- System assessment to determine whether the water supply chain (from source through treatment to the point of consumption) as a whole can deliver water of a quality that meets the above targets;
- Operational monitoring of the control measures in the supply chain which are of particular importance in securing drinking-water safety;
- Management plans documenting the system assessment and monitoring; and describing actions to be taken in normal operation and incident conditions; including upgrade and improvement documentation and communication;
- A system of independent surveillance that verifies that the above are operating properly.

Components 2, 3 and 4 encompass the Water Safety Plan (WSP) that is a new component of the Guidelines. For more information the reader is referred to the Guidelines.

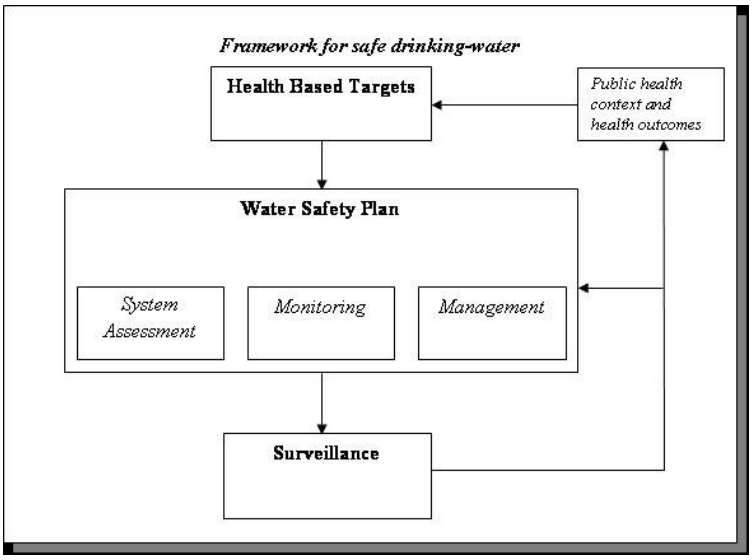


Figure 1. The framework for safe drinking-water. From WHO Guidelines for Drinking Water Quality, 3rd edition, WHO, Geneva.

1.2 SYSTEM ASSESSMENT

Assessment of the ability of the drinking-water system to meet the health-based targets is one of the components of a Water Safety Plan (WSP, Figure 1). System assessment is equally applicable to large utilities with piped distribution systems, and piped and non-piped community supplies, including hand pumps, and individual domestic supplies. Assessment can be of existing infrastructure or of plans for new or upgrading supplies. As drinking-water quality varies throughout the system, the assessment should aim to determine whether the final quality of water delivered to the consumer is able to routinely meet established health-based targets. Understanding source quality and changes through the system requires expert input. The assessment of systems should be reviewed periodically.

This background document aims to give guidance on a System assessment for *Cryptosporidium*, one of the microbial hazards for drinking water safety. *Cryptosporidium* is considered as reference pathogen for the enteric protozoan pathogens (see 1.3). In this document, Quantitative Microbial Risk Assessment is used as tool to quantify the risks associated with *Cryptosporidium* in water supply. It describes the information that water suppliers need to collect to be able to assess the safety of their water supply system from the catchment to the consumer and how this information can be transformed into a quantitative assessment of the safety for the drinking water consumer. By compiling the current state of

the scientific literature on *Cryptosporidium,* this document also serves to validate the System Assessments made by water suppliers. Validation is an element of a System Assessment that is undertaken to ensure that the information supporting the WSP is based on state-of-the art scientific knowledge. This document provides guidance based on (and refers to) the scientific literature on *Cryptosporidium* and QMRA. Water suppliers can refer to this document as state-of-the-science and reference for the QMRA methodology for their System Assessment.
When the QMRA shows that the system is theoretically capable of meeting the health-based targets, the WSP is the management tool that will assist in meeting the targets at all times. If the system is unlikely to be capable of meeting the health-based targets, a programme of upgrading (which may include capital investment and/or training) should be initiated to ensure that the supply will meet the targets in due course. In the interim period, every effort should be made to supply water of the highest achievable quality. Where a significant risk to public health exists additional measures may be appropriate.

1.3 REFERENCE PATHOGENS

In the WHO GDWQ the concept of reference pathogens is introduced: "It is neither possible nor necessary to consider all pathogens in order to design and operate safe drinking-water supplies. Waterborne pathogens vary in size, in their ability to survive in the environment, through different water treatment processes and in the distribution system; they also vary in their infectivity and in the severity of the diseases they cause. In identifying specific pathogens that by their characteristics can represent a group of similar pathogens, it is possible to limit the necessary information and considerations. Such pathogens can provide a reference for developing design and implementation guidelines to meet water quality goals for an entire group of pathogens. In order to protect public health such pathogens should be those within the group that are most difficult to remove or control and that have the largest associated health burden, both on a population and on individual basis. Ideally, there should also be ample high quality data on each aspect of relevance to assessing and managing risks." This implies that, when a water supply system meets the water quality targets for the reference pathogen, it also meets the water quality targets for the group of pathogens that it is considered reference pathogen for.

1.4 WATERBORNE PROTOZOAN PATHOGENS

In the group of parasitic protozoa, several species may be transmitted to humans through the drinking water route. These are: *Entamoeba histolytica*, *Cryptosporidium (primarily C. hominis* and *C. parvum* cattle genotype), *Giardia intestinalis, Toxoplasma gondii, Balantidium coli, Cyclospora cayetanensis,* Microsporidia, *Isospora belli, Naegleria fowleri and Acanthamoeba sp.* For the selection of a reference pathogen for this group, waterborne outbreaks are an important source of information. Outbreaks indicate which pathogens have

been able to break through the multiple barriers and cause disease. Other criteria for the selection are the prevalence and severity of the illness they cause, and the difficulty to control them in water treatment. A practical criterion for the selection of the reference pathogen is the availability of data on waterborne transmission and on the efficacy of control measures. Table 1 summarises the characteristics of the waterborne protozoan parasites. This table aims to give an indication, rather than a quantitative analysis of the different parasites. For more information about the other parasites, the reader is referred to the review books on waterborne disease from Hunter [1997] and Percival *et al.* [2004]. Most of the parasites in table 1 are transmitted through the faecal-oral route, except for the free-living aquatic amoeba *Naegleria, Acanthamoeba* and *Balamuthia. Naegleria fowleri* has occasionally caused PAM in swimmers in recreational surface waters [Cerva & Novak, 1968; Duma *et al.,* 1971; Lares-Villa *et al.,* 1993] or a warm spa [Cain *et al.,* 1981]. In Western Australia, cases have been associated to drinking water from overland mains that were heated by the sun [Robinson *et al.,* 1996]. *Balamuthia mandrillaris* may cause granulomatous amoebic encephalitis (GAE) [Schuster *et al.,* 2003] and the environment (soil) is suggested as the source of infection. No water-related cases are reported.

Acanthamoeba may also cause GAE primarily or exclusively in immunodepressed patients [Martinez & Visvesvara, 1997] and has occasionally caused keratitis in contact lens wearers [Kilvington & White, 1994]. Risk factors are home made saline solution to rinse contact lenses [Visvesvara, 1991] and the initial source of the amoeba was thought to be the drinking water distribution network where these amoeba can live if there is sufficient biofilm on pipe-walls.

From the faecal-orally transmitted parasites, *Entamoeba histolytica, Cryptosporidium hominis* and *C. parvum, Giardia intestinalis* and *Toxoplasma gondii* are the most commonly associated with human illness, with prevalences of 2-50% [Hunter, 1997; Percival *et al.,* 2004]. *Balantidium coli* is also found regularly, esp. in (sub)tropical regions with pig-farming (pigs are the principal animal reservoir), such as the Philippines (prevalence around 1%) [Barnish & Ashford, 1989]. *Blastocystis hominis* is frequently found in both asymptomatic persons and persons with symptoms of an intestinal infection. The epidemiology of this fecal-oral protozoan is not well understood, but no water-related cases are reported.

Most data on *Cyclospora cayetanensis* come from Nepal, Peru, Haiti and Guatemala where *Cyclospora* is endemic [Soave, 1996]. The prevalence is 2-6% in these countries. In industrialised countries, the incidence is generally lower. Microsporidia and *Isospora belli* are found occasionally, primarily or exclusively in immunocompromised (HIV) patients [Cali, 1999; Garcia, 1999].

Waterborne transmission is most frequently reported for *Cryptosporidium, Giardia* and *Entamoeba* [Kramer *et al,* 1996; Barwick *et al.,* 2000 Hunter, 1997]. Compared to *Giardia, Entamoeba, Toxoplasma, Balantidium* and *Cyclospora*, *Cryptosporidium* is the most persistent in the environment, most resistant to chemical disinfection, and smallest in size, so the most difficult to remove by filtration. The high profile this parasite has received in water supply research means that many data are available about this transmission route. *Cryptosporidium* is therefore the pathogen of choice as reference for *protozoan parasites*

that use the faecal-oral route in piped water supplies, both community systems and small (chlorinated) supplies. It is not considered a reference pathogen for:

- *the free-living aquatic protozoa*, because of their different ecology;
- *non-piped, non-chlorinated supplies*, because there are no control challenges that are specific for *Cryptosporidium* in these systems and other enteric pathogens are likely to have a higher health burden in these systems;
- *all faecal-oral pathogens (esp. viruses, bacteria)* that have been shown to cause disease outbreaks through drinking water. Given the differences in health burden, infectivity, nature, size, surface characteristics, resistance to disinfectants, sources, etc.., *Cryptosporidium* cannot be regarded as a reference for all these faecal-oral pathogens, so additional reference pathogens are needed to guide risk management to design safe water supply systems.

Table 1. Characteristics of waterborne protozoan parasites.

Pathogen	**Associated health burden**			**Difficulty to control**			**Data**
	Health symptoms	**Incidence of illness**	**Outbreaks through water supply**	**Persistence in environment**	**Resistance to chemical disinfection**	**Size (µm)**	**Availability for QMRA**
Faecal-oral transmission							
Entamoeba histolytica	Asymptomatic to severe	Common	Many	Moderate	High	10 - 16	Low
Giardia lamblia	Moderate	Common	Many	Moderate	High	9 - 14	High
Cryptosporidium	Moderate	Common	Many	Long	Very high	4 - 6	High
Toxoplasma gondii	Moderate	Common	Few	Long	Very high	10 - 14	Low
Cyclospora cayetanensis	Moderate	Rare	Few	Long	High?	7 - 10	Low
Microsporidia	Moderate	Rare	Uncertain	Long	High?	1 - 4.5	Low
Balantidium coli	Asymptomatic to moderate	Moderate	Very few	Long?	?	45 - 70	Low
Isospora belli	Moderate	Rare	None	Long?	High?	14 - 32	Low
Blastocystis hominis	Asymptomatic to moderate	Common	None	Long?	?	4 - 6	Low
Other route of transmission							
Acanthamoeba	Severe/very severe	Very rare	Few	Lives in water	High for cysts	25 - 40t[*] 10 - 30c	Low
Naegleria fowleri	Very severe	Very rare	One	Lives in water	Moderate High for cysts	10 - 15t[*] 10 - 15c	Low
Balamuthia mandrillaris	Very severe	Very rare	None	Lives in water	?	12 - 60t 6 - 30c	Low

[*] t= trophozoite, c=cyst

2

Hazard identification

2.1 *CRYPTOSPORIDIUM*

2.1.1 Description

Cryptosporidium is a small protozoan parasite that infects the microvillous region of epithelial cells in the digestive and respiratory tract of vertebrates. It is an obligate intracellular parasite of man and other mammals, birds, reptiles and fish. It requires its host to multiply. Environmentally robust oocysts are shed by infected hosts into the environment. These oocysts can survive the adverse conditions on the environment for months until ingested by a new suitable host. In the new host, the life cycle starts again and multiplication occurs, using resources of the host. The parasite has been first described in mice in 1907 [Tyzzer, 1907], but was not recognised as a causative agent for human illness until 1976 [Nime *et al.,* 1976; Meisel *et al.,* 1976]. It was first associated with disease in severely immunocompromised individuals, esp. AIDS patients with low CD4-counts, but is now also recognised as widespread, general pathogen of immunocompetent humans.

2.1.2 Taxonomic position

Cryptosporidium is part of the Apicomplexa, Cryptosporidiidae and has been classified as member of the group of eimeriid coccidia, a diverse group of parasitic protozoa. Recent taxonomic studies place *Cryptosporidium* as a clade separate from the coccidia. A study on the 18S rRNA gene indicated a closer relation to the gregarines [Carreno *et al*., 1999]. This would also explain why *Cryptosporidium* has several features that separate it from the other coccidia: infection of the host is confined to the apical region of the epithelial cells, the small size of the oocysts, the formation of both thick- and thin-walled oocysts and the insensitivity to anti-coccidial agents. Further understanding of the relation to the gregarines is very important for understanding its ecology and waterborne transmission [Ryan & Xiao, 2003]. The gregarines are parasites to freshwater invertebrates and cross-reaction of *Cryptosporidium* antibodies to gregarines can occur [Bull *et al.,* 1998]. Even multiplication of *Cryptosporidium* in freshwater hosts could occur and development of extracellular life-cycle stages in water has been suggested [Boxell *et al.,* 2004].

Until the mid-1990's, several species of *Cryptosporidium* had been described. The species description was based primarily on morphology and host specificity. *C. parvum, C. muris, C. felis* and *C. wrairi* were identified as species that infect mammals, *C. baileyi* and *C. meleagridis* infect birds, *C. serpentis* and *C. saurophilum* infect reptiles and *C. nasorum* tropical fish. *C. parvum* was the primary species isolated from infected humans. *C. parvum* was isolated from 152 species of mammals [Casemore *et al*, 1997; Fayer *et al.,* 2000] and cross-transmission studies indicated that *C. parvum* isolates can be transmitted from humans to animals and between different animals. Human cases of cryptosporidiosis were associated with animal contact to humans. This gave the impression that the host-range of *C. parvum* was very broad, and hence many animals served as reservoir for *Cryptosporidium* that could infect man.
Molecular taxonomy, based on several markers (such as the 18S rRNA gene, the *Cryptosporidium* oocyst wall protein (COWP) gene and TRAP-2 gene), have now shown that the taxonomy is more complex. Morgan *et al*. [1999] have reviewed the taxonomic information and have seen considerable genetic heterogeneity between isolates of *Cryptosporidium* from different vertebrate species. They have proposed a revised taxonomy, suggesting that host specific genotypes occur within the species of *C. parvum*: a human genotype (H-type or type 1), a cattle genotype (C-type or type 2), a ferret genotype, a pig and marsupial genotype, a dog genotype and two genotypes that were classified as distinct species: *C. wrairi* and *C. felis*. The H-type was recently renamed as a new species *C. hominis* [Morgan-Ryan *et al.*, 2002], which appears to be specific to humans although there are reports of *C. hominis* infection in gnotobiotic piglets [Widmer *et al.,* 2000] and in the dugong [Morgan *et al.,* 2000]. The dog genotype was renamed *C. canis* [Fayer *et al.,* 2001] and also the pig genotype(s) is proposed as separate (pig-specific) species *Cryptosporidium suis* [Ryan *et al.,* 2003]. Other genotypes of *Cryptosporidium* have been identified in wildlife

[Fayer *et al*., 2000], [Perz & LeBlanq, 2001; Morgan *et al.,* 1998], but these have not been found in humans [Xiao *et al*., 2004].

Currently, 13 of the parasite species are regarded as valid [Table 2, Ryan *et al*., 2003]. Several other *Cryptosporidium* genotypes have been described from mammals, birds and reptiles which need further characterisation to determine the species status.

Table 2 Cryptosporidium species

Species	**Hosts**	**Isolated from human cases**	**Implicated in waterborne outbreak**
C. hominis	Humans	Frequently	Yes
C. parvum	Mammals	Frequently	Yes
C. meleagridis	Turkey, humans	Occasionally	No
C. muris	Rodents, ruminants	Very occasionally	No
C. andersoni	Cattle, camel	No	No
C. felis	Cats	Very occasionally	No
C. canis	Dogs	Very occasionally	No
C. wrairi	Guinea pigs	No	No
C. baileyi	Gallinaceous birds	One report	No
C. galli	Birds	No	No
C. serpentis	Snakes	No	No
C. saurophilum	Lizards	No	No
C. molnari	Sea bass, sea bream	No	No

Infection in immunocompetent human hosts is predominantly caused by *C. hominis* and the cattle genotype of *C. parvum*. [McLauchlin *et al*., 2000]. Other *Cryptosporidium* species have been reported to infect humans, but less frequently (*C. meleagridis, C. felis* and *C. canis*) or very occasionally (*C. muris, C. andersoni* and the pig genotype of *Cryptosporidium*) [Pieniazak *et al.*, 1999; Morgan *et al*., 1999b; 2000; Pedraza-Días *et al.*, 2000; Xiao *et al* 2001; Yagita *et al.,* 2001; Gatei *et al*., 2002; Tangtip & Jongwutiwes*,* 2002; Xiao, 2004].

Interestingly, studies from Australia and North America indicate that *C. hominis* is most prevalent in humans [Morgan *et al.,* 1998; Xiao *et al.,* 2002; Ong *et al.,* 2002], while studies in Europe indicate that the cattle genotype of *C. parvum* is most prevalent in humans [McLauchlin *et al.,* 2000; Lowery *et al.,* 2001; Fretz *et al.,* 2001]. The reasons for this discrepancy are not clear. A recent UK-survey of 5001 faecal specimens of confirmed human *Cryptosporidium* infections in the UK, *C. hominis* was identified in 50% of the specimens, *C. parvum* cattle genotype in 45%, 4% could not be identified and *C. meleagridis* was found in 0.6%. *C. felis* and *C. canis* were found in 3 and 1 specimens respectively [Chalmers *et al.,* 2003].

The taxonomy of *Cryptosporidium* is still under development. Understanding the taxonomic position and the differentiation of the species and subtypes is not only relevant to

evolutionary biology, but also to understanding the sources and environmental transmission of human cryptosporidiosis.

2.1.3 Life cycle

Infected hosts shed oocysts, the environmentally resistant transmission stage of the parasite, with their faeces [Fayer & Ungar, 1986; Fayer *et al*., 1997]. These oocysts are immediately infectious and may remain in the environment for very long periods without losing their infectivity, due to a very robust oocyst wall that protects the four sporozoites against physical and chemical damage. When the oocyst is ingested by a new host, the suture in the oocyst wall opens (excystation), triggered by the body temperature and the interaction with stomach acid and bile salts. Four motile sporozoites are released into the small intestine of the host and they infect the epithelial cells of the small intestine, mainly in the jejunum and ileum. The parasite infects the apex of the epithelial cells and resides beneath the cell membrane of the epithelial cells but outside of the cytoplasm. The sporozoites transform into several life stages in an asexual (merogony) and a sexual reproduction cycle (gametogony). The oocysts are the result of the sexual reproduction cycle. Oocysts of *C. hominis/C. parvum* are spherical with a diameter of 4-6 µm. Thick- and thin-walled oocysts are formed. The thin-walled oocysts may excyst within the same host and start a new life cycle (autoinfection). This may lead to a heavily infected epithelium of the small intestine, resulting in malabsorptive or secretory diarrhoea. The thick-walled oocyst is excreted with the faeces and is environmentally robust.

2.1.4 The disease

A description of the health effects in immunocompetent and immunocompromised individuals is given in chapter 5.2.

2.1.5 Prevalence

In stool surveys of patients with gastro-enteritis, the reported prevalence of *Cryptosporidium* is 1-4% in Europe and North America and 3-20% in Africa, Asia, Australia, South and Central America [Current & Garcia, 1991]. Peaks in the prevalence in developed countries are observed in the late summer [van Asperen *et al*., 1996] and in spring [Casemore, 1990]. In industrialised countries, the prevalence is high in children under 5 years of age and in young adults. In developing countries, infection is common in infants less than 1 year, but is rarely seen in adults.
Asymptomatic carriage, as determined by stool surveys, generally occurs at very low rates in industrialised countries (<1%) [Current & Garcia, 1991], although in day care centres higher rates have been reported [Lacroix *et al*., 1987; Crawford & Vermund, 1988; Garcia-Rodriguez *et al*., 1989]. Routine bile endoscopy suggests a higher asymptomatic prevalence:

13% of non-diarrhoeic patients were shown to carry *Cryptosporidium* oocysts [Roberts *et al*., 1989]. High rates of asymptomatic carriage (10-30%) are common in non-industrialised countries [Current & Garcia, 1991]. Seroprevalence rates are generally higher than faecal carriage rates, from 25-35% in industrialised countries up to 68-88% in Russia [Egorov *et al.,* 2004] and 95% in South America [Casemore *et al*, 1997]. Seroprevalence rates increase with increasing age [Zu *et al*., 1992; Kuhls *et al*., 1994; Egorov *et al.,* 2004] and are relatively high in dairy farmers [Lengerich *et al*., 1993] and day care centre attendants [Kuhls *et al*., 1994]. Two city studies in the USA showed that people that consumed treated surface water were more likely to show seroconversion during the study period than the people that consumed well-protected groundwater [Frost *et al.*, 2001; 2002; 2003]. During the months of the study, a significant proportion of the population exhibited seroconversion (also in the groundwater cities), indicating that *Cryptosporidium* infections may be relatively common. Illness rates were not increased in the cities supplied with surface water, so, although infections were more common, illness was not. The more intense serological response in the residents of the surface water cities could indicate an increased level of protection from illness. Human feeding trials also indicated a protective effect of a prior infection to illness after low dose exposure, but not against high dose exposure [Chappell *et al.,* 2004]. Both in the USA and in Russia, consumption of drinking water from shallow wells was correlated to a high seroprevalence [Frost *et al.,* 2003; Egorov *et al.,* 2004].

2.1.6 Routes of transmission

The majority of human infections are caused by *C. hominis* and the cattle genotype of *C. parvum*. Other *Cryptosporidium* species that occasionally infect immunocompetent humans are *C. meleagridis, C. felis* and *C. canis.* Species that have been reported only in immonocompromised individuals are *C. muris/andersoni* (evidence on species not conclusive) and a cervine and pig genotype [Xiao, 2004]. It is likely that other species or genotypes will be found in (immunocompromised) humans in the future, but these will probably account for only a (very) small fraction of human infections.

As *C. hominis* and the cattle genotype of *C. parvum* account for the vast majority of human infections, the sources of these species are the predominant reservoirs of human cryptosporidiosis. Humans are the only significant source of *C. hominis* and humans and ruminants are the predominant sources of the cattle genotype of *C. parvum* [Xiao *et al.,* 2003]. The cattle genotype of *C. parvum* has been found in other mammals, but infected humans, cattle and sheep shed oocysts in very high numbers, especially when infected in infancy, which probably contribute most to the environmental contamination. Transmission occurs through direct or indirect contact with faeces of these shedders. Outbreaks illustrate the different routes: person-to person spread in institutions, animal contact during farm visits, contact with recreational waters, swimming pool visits, municipal drinking water and food.

Human-to-human

Person-to-person transmission is a common route, as illustrated by outbreaks in day-care centres [Fayer & Ungar, 1986; Casemore, 1990; Cordell & Addiss, 1994] and the spread of these outbreaks in the households of the attending children. Patient-to-patient or patient-to-health care staff transmission may occur in hospitals [Casemore *et al.,* 1994]. Also sexual practices that imply oro-anal contact yield a high risk for exposure to *Cryptosporidium*.

Case control studies show that major risk factors are household contacts with people (esp. children) with diarrhoea [Robertson *et al.,* 2002; Hunter, 2003]. Another risk factor that is generally found in these studies is foreign travel, esp. to countries with a higher prevalence of cryptosporidiosis.

Animal-to-human

Zoonotic transmission of *Cryptosporidium parvum* is well documented. There are various reports of outbreaks or cases of cryptosporidiosis in school children or students after exposure to calves or lambs [Casemore, 1990; Casemore *et al.,* 1997]. Occupational exposure to infected animals (mainly calves) has also resulted in human infection [Current, 1994; Casemore *et al.*, 1997]. The recent genotypic evidence suggests that only the cattle genotype of *C. parvum* is capable of zoonotic transmission [Sulaiman *et al.,* 1998], but this genotype has been found in many host species (humans, cattle, pigs, sheep). The high prevalence of the *C. parvum* in cattle and sheep and the high numbers of oocysts shed by infected animals (esp. newborns) make cattle and sheep important sources of environmental contamination with *Cryptosporidium* oocysts that are able to infect humans.

Indirect evidence indicates that contact with horses and contact with horse manure are risk factors for cryptosporidiosis [Casemore, 1990]. However, only immonosuppressed horses have been shown to carry the cattle genotype of *C. parvum*, while immunocompetent horses carry a unique horse genotype.

Cryptosporidium meleagridis may infect humans and is found in turkeys worldwide [McDougald, 1998]. Outbreaks of avian cryptosporidiosis have been reported in turkey farms, and these may be the main source of environmental contamination with *C. meleagridis.*

Also pet animals can be infected with *Cryptosporidium* oocysts, but these do not appear to be an important source of human infection [Casemore *et al*., 1997; Glaser *et al*., 1998]. The species found in cats (*C. felis*) and dogs (*C. canis)* are occasionally found in immunocompromised humans [Pedraza-Dias *et al.,* 2001]. Hence, cats and dogs should be considered as a potential source of infection to humans if they are immunocompromised. The prevalence of *C. felis* in cats is 2.4 – 8.2% and the prevalence of *C. canis* in dogs is 1.5 – 45% [Olson *et al.,* 2004].

The role of wildlife as a source is less clear. *Cryptosporidium* sp. have been identified in many species of wildlife, but genotyping studies generally identify the isolates from wildlife

as unique genotypes [Olson *et al.,* 2004]. This indicates that wildlife species are host to specific *Cryptosporidium* species that are not found in other animals including humans. All these animals shed oocysts into the environment, contributing to the total *Cryptosporidium* load of drinking water sources. Conventional detection techniques do not discriminate well between the different species and genotypes. Therefore, genotyping of environmental isolates is important to determine the presence of genotypes that are pathogenic to humans, especially in more pristine environments.
Waterfowl and insects have been suggested as vectors of *C. parvum*, where the animals pick up oocysts from contact with human or cattle faeces and may deposit them again in water or on food [Graczyk *et al.,* 1996, 1997, 1999, 2000; Szostakowska *et al.,* 2004]. The flies may be important for food borne transmission. The significance of waterfowl in contamination of watersheds with oocysts that may infect humans is unknown.

Water
Indirect person-to-person or zoonotic transmission may occur by contamination of water used for recreation or drinking, swimming pools or food. Surface water becomes contaminated through the discharge of untreated and treated sewage and run-off of manure. The relative significance of these sources may differ between watersheds. Large rivers and lakes often receive both agricultural run-off and treated and untreated domestic wastewater.
As a result, *Cryptosporidium* oocysts of various genotypes are ubiquitous in surface waters throughout the world. Reported concentrations generally range from 0.01-100 per litre. These concentration data are not corrected for the (low) recovery of the detection method, so the actual concentrations may be more than tenfold higher. Higher concentrations are found in urbanised or agricultural waters than in pristine waters [LeChevallier *et al*., 1991; Rose *et al*., 1991]. 12% of groundwater supplies in the US were contaminated with *Cryptosporidium* and/or *Giardia* [Hancock *et al*., 1997], mostly in infiltration galleries and horizontal wells (impacted by surface water contamination).
Although water is probably not the most important route of transmission, many waterborne outbreaks of cryptosporidiosis have been described, some of which are very large. The largest outbreaks of cryptosporidiosis were attributed to contaminated drinking water, both from surface water and groundwater sources [Craun, 1992; Mackenzie *et al*., 1994; Hunter, 1997; de Jong & Andersson, 1997; Glaberman *et al.,* 2002; Guyonnet & Claudet, 2002]. Outbreaks have also been associated with exposure to recreational water and swimming pools [Joce *et al*., 1991; Sorvillo *et al., 2002;* MacKenzie *et al*., 1995; van Asperen *et al*., 1996; Anon, 1998; Kramer *et al*., 1998; Puech *et al.,* 2001; Nichols *et al.,* 2004]. In several case control studies swimming in recreational water and/or a swimming pool was identified as risk factor for cryptosporidiosis [Puech *et al.,* 2001]. Drinking water from community supplies was not identified as risk factor [Sorvillo *et al.,* 1994; Khalakdina *et al.,* 2003; Robertson *et al.,* 2004]. Sea water may contain *Cryptosporidium* [Johnson *et al.,* 1995; Fayer *et al.,* 1998], but has not been implicated in outbreaks.
In several waterborne outbreaks the *Cryptosporidium* isolates have been genotyped and both *C. hominis* and the cattle genotype of *C. parvum* have been implicated as causative agents. In

most outbreaks, only one genotype was identified, but in some outbreaks both genotypes have been found [Xiao *et al.,* 2004; Mathieu *et al.,* 2004].

Food
Outbreaks have occurred through consumption of contaminated food (raw milk and meat, farm-made apple cider, fermented milk, salads, raw vegetables)[Casemore *et al*., 1997]. Food can be contaminated by infected food handlers [Quinn *et al.,* 1998; Quiroz *et al.,* 2000], irrigation with contaminated water or manure. *Cryptosporidium* has been found in shellfish, such as oysters [Fayer *et al.,* 1998; 2003; Schets *et al.,* 2002], and on raw vegetables suggesting these could be routes of transmission as well. Interestingly, eating raw vegetables was a protective factor against cryptosporidiosis in a case-control study in the UK [Hunter, 2003]. This could be related to repeated exposure through this route and build-up of protective immunity, but this is not proven.

2.2 CHARACTERISTICS RELATING TO WATERBORNE TRANSMISSION

Several characteristics of *Cryptosporidium* facilitate waterborne transmission. These are outlined below.

2.2.1 Extreme resistance to chemical disinfection

Disinfection with chlorine has always been an important barrier for waterborne pathogens. The high resistance of *Cryptosporidium* oocysts against chlorine disinfection [Korich *et al*., 1990; Smith *et al*., 1990; Ransome *et al*., 1993] renders this process ineffective for oocyst inactivation in drinking water treatment. Chlorine dioxide is slightly more effective, but still requires a high CT product (measure of disinfectant dose: (residual) concentration of disinfectant C x contact time T) of 75 - 1000 mg.min l^{-1} for 99% inactivation of oocysts [Korich *et al*., 1990; Chauret *et al*., 2001].
Ozone is the most potent chemical oocysticide: at 20°C, the CT for 99% inactivation of *C. parvum* oocysts is 3.5 mg.min.l^{-1} [Finch *et al*., 1993]. The effectiveness of ozone reduces at lower temperatures and the CT values required for inactivation of oocysts at low temperatures are high. CT values are limited, however, since high CT's can give rise to formation of high concentrations of (geno)toxic by-products.
Exposure of *Cryptosporidium* oocysts to multiple disinfectants in succession has been shown to be more effective than was to be expected from both disinfectants alone [Finch *et al*., 1994; Liyanage *et al*., 1997] and synergism between disinfection and environmental stress during sand filtration has also been observed [Parker *et al*., 1993]. The multiple stresses that (oo)cysts encounter in the environment and during treatment might limit the infectivity of (oo)cysts.

Although older literature suggests that UV systems have a limited effect on *Cryptosporidium* viability, more recent work shows that this was due to the use of in vitro viability assays that over-estimate infectivity. Clancy *et al.* [1998], using animal infectivity, showed that medium-pressure UV is very effective against *Cryptosporidium*; they obtained 99.98% inactivation at UV-doses as low as 19 mJ/cm^2. Many successive studies have shown that oocysts are sensitive to low or medium pressure UV [Craik *et al.*, 2001; Shin *et al.,* 2001; Morita *et al.,* 2002; Clancy *et al.,* 2002; Rochelle *et al.,* 2004].

More detail on disinfection of *Cryptosporidium* is given in chapter 4.

2.2.2 Persistence in the environment

Oocysts can survive for months in surface water [Robertson *et al.*, 1992; Chauret *et al.*, 1995; Medema *et al.*, 1997]. Under natural conditions, the die-off rate of *Cryptosporidium* oocysts in water is 0.005-0.037 ^{10}log-units per day. Oocysts also survive well in estuarine waters (over 12 weeks at 20C and a salinity of 10), but less in seawater (4 weeks at salinity of 30 ppm) [Fayer *et al.,*1998].
In the older studies, survival was monitored with *in vitro* assays such as excystation or dye exclusion. The longevity of oocysts in fresh water has been confirmed in studies that use enumeration by cell culture infectivity; King *et al.* [2005] showed inactivation rates of 0.095, 0.048, 0.011 and <0.01 ^{10}log/day of *C. parvum* oocysts in freshwater at 25, 20, 15 and 4°C respectively.
Long survival (120 days) of oocysts in soil has also been reported [Kato *et al.,* 2004]. Oocysts in soil do not survive well when artificially frozen/thawed in the soil [Kato *et al.,* 2002] or under field conditions in Norwegian soil [Robertson & Gjerde, 2004].

2.2.3 Small size

Compared to other protozoan parasites, the oocysts of *Cryptosporidium* are small (4-6 µm). Due to their smaller size, the are less efficiently removed during soil passage, in bank filtration and in rapid or slow sand filtration in drinking water treatment. However, compared to bacteria (~1 µm) and esp. viruses (20-60 nm), oocysts of *Cryptosporidium* are large.

Although the state in which (oo)cysts occur in water (suspended or attached to particles) is relevant for water treatment (sedimentation, filtration), and oocysts readily attach to particles in sewage effluent [Medema *et al.*, 1998], little information is available as yet on the significance of these factors in the environmental ecology of (oo)cysts. Dai & Boll [2003] reported no attachment of oocysts to loam or sand particles in batch experiments.

2.2.4 High infectivity

The infectivity of oocysts is high. Extrapolation of the dose-response data [Chappell *et al.,* 1999, (extensively discussed in chapter 5)] indicates that ingestion of a single oocyst gives a discrete probability of infection. The occurrence of waterborne outbreaks with high attack rates substantiates this. The reviews of *Cryptosporidium* concentrations in drinking water during outbreaks [Haas & Rose, 1995; Craun *et al.,* 1998] suggests that consumers ingest only one to a few oocysts per day. The infectivity of oocysts varies between isolates. A comprehensive analysis of the dose-response data from volunteer studies with the different isolates is given in chapter 5.
Other waterborne pathogens exhibit an even higher infectivity than *Cryptosporidium*; this includes several viruses (rotavirus, enteroviruses, Norovirus (?)), *Giardia* and *Campylobacter* [Teunis *et al.,* 1996].

2.2.5 Human and livestock sources

As discussed in 2.1.5, the majority of human infections are caused by *C. hominis* and *C. parvum*. *C. hominis* is transmitted between humans and *C. parvum* is transmitted between humans and from other mammals (esp. ruminants) to humans. *Cryptosporidium* sp. has been isolated from cattle worldwide, mostly from diarrheic newborn calves. The prevalence in diarrheic calves is very high. A wide range of prevalences have been reported in calves (5-100%) [Angus, 1990; Casemore *et al.*, 1997; Olson *et al.,* 2004], but this is probably due to differences in detection methods applied and the age of the calves sampled. 90 -100% of herds may be infected [Medema *et al.,* 2001]. Clinical infection occurs primarily in the newborn calves, which may shed more than 10^{10} oocysts/day. Prevalence is lower in adult cattle.
Sheep are also hosts of *C. parvum.* Lambs of 1 – 2 weeks old are most commonly infected and in some cases mortality can be high [Angus, 1990]. Reported prevalence in Spain was highest during spring, where 40% of lambs (90% of farms) shed *Cryptosporidium* sp., compared to 8% in autumn (40% of farms) [Matos-Fernandez *et al.,* 1994]. The same study reported high prevalence (70%) in goats. Also for goats, prevalence is high in kids of 5-15 days [Angus, 1990]. Olson *et al.* [2004] reviewed published prevalence data for sheep: 10 – 78%; and goats: 28 – 100%.
Cryptosporidium infection has been described in horses, again mainly in very young animals [Xiao & Herd, 1994[a]]. Prevalence rates were 17-31%, but these were probably the horse genotype that is not found in humans.
The close contact with cattle and sheep make the risk of transmission high. The high density of cattle and sheep in watersheds and the excretion of high numbers of oocysts make these animals important sources of environmental contamination, which have been implicated in several waterborne outbreaks of cryptosporidiosis.

2.2.6 Oocyst shedding in high numbers

During acute infection, oocysts can be found in high numbers in the faeces of the host. This is facilitated by auto-infection of the host (see 2.1.2). At the peak of the infection, infected humans shed up to 10^{5-7} per gram faeces [Chappell *et al.,* 1999]. Rose *et al.* [1986] and Madore *et al.* [1987] reported an average number of 5300 *Cryptosporidium* oocysts per litre in untreated sewage water and a range of 850-14,000 oocysts per litre. However, only a small number of samples were taken (n = 4). Rose *et al.* [1996] found 67% of the raw sewage samples in St. Petersburg, Florida positive, with an average concentration of 1500 per litre (maximum 12000 per litre); 42% of the effluent samples were positive with an average concentration of 140 per litre (maximum 1100 per litre). The removal efficiency rate was 91% (1.0 log) for *Cryptosporidium* for the combined processes. In a wastewater treatment plant in Israel, the number of *Cryptosporidium* oocysts varied from 300 to7700 per litre. The purification efficiency of 93% resulted in 50 oocysts/l in the effluent [Nasser & Molgen, 1998]. In a Canadian study by Chauret *et al.* [1999] 54 effluent samples were taken at the Ottawa-Carleton wastewater treatment. The numbers of *Cryptosporidium* were 50 per litre. In the Netherlands, 2 wastewater treatment plants were investigated [Medema *et al.,* 2001]. Geometric mean oocyst concentration in raw and sedimented sewerage (pre clarifier) were 540 and 4650 per litre and in effluent of these biological treatment systems were 17 and 250 per litre respectively.

Several studies show that infected cattle, especially newborn calves, sheds high numbers of oocysts [Anderson & Bulgin, 1981; Current, 1987; Casemore *et al.*, 1997]. In newborn calves, excretion of oocysts usually occurs after 7 days and peaks around 14 days. At the peak of the infection, 10^{6-7} oocysts per gram faeces are excreted. Several authors have studied the shedding patterns of calves quantitatively. Xiao & Herd [1994[b]] found oocyst concentrations of 10^{4-7}/gram faeces in calves of 1-6 weeks. Medema *et al.* [2001] found oocysts in 90% of the veal calf herds when manure of 1-6 week-old calves was sampled. The average concentration of oocysts was 5.2×10^4 per gram (range $0 - 1.9 \times 10^5$ /gram). Both the prevalence and the concentration of oocysts declined as the calves grew older, respectively to 20% and 2.6×10^3/gram at calves of 26 weeks [Medema *et al.*, 2001]. They estimated the annual emission of oocysts by all veal calves in the Netherlands to be 1.2×10^{15} oocysts. Svoboda *et al.* [1997] found a median concentration of 3×10^6 oocysts/gram of calve faeces (range $0 - 1.3 \times 10^8$/gram), resulting in a daily oocyst excretion of $>10^{10}$. Adult cattle showed much lower concentrations of oocysts (0.5 – 45/gram), resulting in a daily excretion of 7×10^5 oocysts. Scott *et al.* [1994] found somewhat higher oocyst-concentrations in adult cattle: 90/gram (range 25-18000/gram). There was no apparent relation with calving. In contrast, Medema *et al.* [2001] did not find *Cryptosporidium* in manure of adult dairy cattle. Fayer [2004] and Olson *et al.* [2004] showed that calves of 1 – 4 weeks predominantly shed *C. parvum,* while older calves were infected primarily by *C. andersoni.* So, young animals are the principal source of zoonotic *C. parvum*. This is important for management of animal farming in watersheds; ensuring that the newborns and

their manure are kept away from water sources may reduce the risk of waterborne transmission considerably.
Slurry from calve housing contains oocysts; Medema *et al.* [2001] found an average concentration of 7500 oocysts (range 6100-9800)/gram. Survival of oocysts in slurry was less than 4 weeks at 20°C; higher survival rates were observed at 4°C [Svoboda *et al.,* 1997].
Shedding of *C. parvum* by other farm animals (sheep, goat, horse) does occur, but is less well studied. Symptomatic infection is also here more common in infant than in adult animals.

2.2.7 No maturation required

Unlike other coccidian parasites, *Cryptosporidium* oocysts do not require a period of maturation of the oocysts after shedding with faeces. They are immediately able to infect a new host.

3

Problem formulation

3.1 IDENTIFICATION OF HAZARDOUS EVENTS

Like many of the waterborne pathogens *Cryptosporidium* is an intestinal pathogen and is transmitted by the faecal-oral route. Many of the hazardous events that can be identified for *Cryptosporidium* are identical to those for other enteric pathogens, such as *Giardia*, but also enteric bacteria (i.e. *Campylobacter*) and viruses (i.e. Noroviruses or Hepatitis A or E viruses), since all of these pathogens originate from faecal contamination. On the other hand, *Cryptosporidium* has characteristics that may results in a relatively high risk of disease in the case of a hazardous event results. These are particularly its extreme resistance to chemical disinfection and long survival in the aquatic environment (see chapter 2).
To identify hazardous events, drinking water outbreaks are an important source of general information on hazardous events leading to waterborne transmission of *Cryptosporidium* (paragraph 3.2). Site-specific information on hazardous events can be obtained from a sanitary survey and from historical monitoring data (paragraph 3.3).
Paragraph 3.4 describes how the information on hazardous events can be used in QMRA.

3.2 LESSONS FROM DRINKING WATERBORNE OUTBREAKS

Many waterborne outbreaks of cryptosporidiosis have been reported in industrialised countries [MacKenzie *et al*., 1994; Hunter, 1997; 2004; Bouchier, 1998; Craun *et al.,* 1998]. The first reported human outbreak of cryptosporidiosis due to contaminated water supplies occurred in Texas in 1984 in conjunction with a Norwalk virus outbreak [D'Antonio *et al*., 1985]. The water source was an artesian well and was suspected of being contaminated with sewage. Disinfection by chlorination was the only treatment and although adequate to control coliform bacteria, it was apparently insufficient in controlling Norwalk virus and *Cryptosporidium*. A second outbreak in Carrollton, Georgia (USA) occurred in January 1987, where over 13,000 people were affected [Hayes *et al*., 1989]. The Carrollton drinking water supply underwent conventional treatment, including coagulation, sedimentation, rapid sand filtration, and disinfection. Subsequent investigations revealed no violations for coliform or turbidity levels. At the same time, an outbreak in the UK was reported that was caused by contamination of the distribution network through contamination of a break-pressure tank, which was enhanced during rainfall [Smith *et al.,* 1989]. Again, the water complied with the coliform standard. These first waterborne outbreaks instructed scientists that:

- *Cryptosporidium* could be transmitted by municipal drinking water systems and cause large outbreaks;
- Systems with chlorination only without filtration were especially vulnerable;
- Outbreaks could occur through drinking water that met the coliform and turbidity standards.

In 1991 an outbreak occurred in the Swindon/Oxfordshire area of the UK through conventionally treated (coagulation/filtration and disinfection) drinking water [Richardson *et al.,* 1991]. In this case, recirculation of filter backwash water caused an increased challenge of the treatment systems with *Cryptosporidium* oocysts that broke through the filters. In April 1993, the largest North American outbreak of cryptosporidiosis was described as apparently affecting some 403,000 people in Milwaukee, Wisconsin and as being caused by cattle faeces in surface water passing through the conventional treatment plant just after a coagulant change-over [MacKenzie *et al*., 1994]. Since that time both the true size and sources of the outbreak have been questioned, with molecular epidemiological evidence pointing to a human rather than a cattle source [Sulaiman *et al.,* 1998] and the true size of the outbreak possibly being exaggerated by orders of magnitude through over-reporting bias [Hunter and Syed, 2001]. These and many other outbreaks informed scientists that conventional treatment systems also can be vulnerable to outbreaks when the coagulation and filtrations systems are not carefully operated and maintained.

In 1998 Sydney experienced a succession of *Cryptosporidium* contamination events. A combination of early detection in samples by the monitoring laboratory, subsequent boil water alerts issued to consumers, and the possibility that oocysts in the supply system were not viable or counts overestimated may have been the reason that no cases of cryptosporidiosis were traced to drinking water during these three events [McClellan, 1998;

Allen *et al*. 2000; Clancy, 2000, 2001; Hawkins *et al.* 2000; 2001]. The incidents did result in a large body of research into the origin of the contamination events. This has lead to the understanding of the transport of *Cryptosporidium* in reservoirs following rainfall events. In this case, the heavy rainfall after a period of draught caused relatively cold floodwater to enter and fill the reservoir (Lake Burrangorang). The thermal stratification of the water in the reservoir caused the colder floodwater to flow along the lake bottom and reached the dam with the off take within days in stead of months. This flow caused an internal wave in the reservoir that hit the off take on several subsequent days, leading to relatively high *Cryptosporidium* counts in the water that entered treatment [Hawkins *et al.,* 2000; Cox *et al.*, 2004].
In the summer of 2002, increased counts of *Cryptosporidium* in treated water (found in the statutory monitoring) led to a boil water alert for Glasgow and Edinburgh. Also here, no increase in the number of gastro-enteritis cases was observed [Healthstream, 27 sep. 2002].

Waterborne outbreaks have indicated the water supply systems that are at risk of *Cryptosporidium*. Since chlorination or chloramination is not effective, unfiltered supplies are at risk and catchment protection is important in management of this risk. Conventional supplies are at risk if water treatment is compromised, especially incombination with a peak contamination event in source water. In addition, several outbreaks through (karst) groundwater supplies indicate that groundwater can be a source of cryptosporidiosis, despite being usually regarded as surface water pathogen. Several outbreaks are associated with ground water under the direct influence of surface water [Willcocks *et al.,* 1998; Bouchier, 1998; Bergmire-Sweat *et al.,* 1999]. Bank filtration systems are part of these groundwaters. The outbreaks show that even deep boreholes can be affected by surface water ingress through fissures in the soil. At the Brushy Creek outbreak, the distance between well system and creek was over 400m [Bergmire-Sweat *et al.,* 1999]. Other means of groundwater contamination that have been associated with outbreaks of cryptosporidiosis is livestock manure (cattle, sheep) near well-heads but also other contamination sources from above (or in) ground (sewers, manure, manure deposits, sludge deposits etc.) *Cryptosporidium* contamination may arise. In general, soils with fractures or fissures are vulnerable (rock, chalk), but also freatic aquifers in more fine-structured soils (sand, gravel) are at risk. Heavy rainfall events are risk events, since they promote rapid transport of contaminations through the soil.

Several authors have reviewed the causes of outbreaks through drinking water [Smith & Rose, 1990; 1998; Badenoch, 1990; 1995; Hunter, 1997; Craun *et al.*, 1998; Bouchier, 1998; Hrudey & Hrudey, 2004] and have made recommendations for optimising water treatment practice (see Box 1 & 2). In a significant number of these outbreaks, the drinking water that was implicated as the cause of the outbreak complied with the WHO-guidelines for *Escherichia coli* levels and turbidity [Craun *et al*., 1998]. In most outbreaks, deviations from normal raw water quality or treatment operation could be identified. However, in a drinking waterborne outbreak in Las Vegas, no abnormalities in operation or water quality (raw or treated) were detected [Goldstein *et al*., 1996].

Box 1.
Badenoch [1995] recommendations for water treatment practices:

To minimise the risk of cryptosporidial oocysts passing into public water supplies, water companies should pay partiular attention to the following:

i. the operation of rapid filters should avoid sudden surges of flow which may dislodge retained deposits;
ii. rapid filters should not be restarted after shutdown without backwashing;
iii. after cleaning, slow sand filters should not be brought back into use without an adequate "ripening period";
iv. by-passing of part of the water treatment process should be avoided.

The information on the events/errors that led to these outbreaks can be used for the identification of hazards. A common thread of many of the reported outbreaks and contamination events is that the disinfection and filtration systems were thought to have been inadequate to prevent contamination, at least in their operational state at the time of contamination. It should be kept in mind however, that the information that is disseminated from outbreak studies can be biased. The question about who is responsible (and may encounter legal actions) for the outbreak has become more and more significant, and this may influence the information that is released.

Drinking-waterborne outbreaks of cryptosporidiosis have been caused by contamination of the source water due to heavy rainfall or melting snow [Richardson *et al*., 1991; MacKenzie *et al*., 1994; Curriero *et al.,* 2001] or to sewage contamination of wells [d'Antonio *et al*., 1985; Kramer *et al*., 1996], inadequate treatment practices [Richardson *et al*., 1991; Craun *et al*., 1998] or treatment deficiencies [Badenoch, 1990; Leland *et al*., 1993; Craun *et al*., 1998] or combinations of these factors [MacKenzie *et al*., 1994]. Also, leakage or cross-connections in the distribution system have caused outbreaks of cryptosporidiosis [Craun, 1992; de Jong & Andersson, 1997; Craun *et al*., 1998; Endo *pers. comm.*].

Box 2.
Selected Bouchier [1998] recommendations for water treatment.

Water utilities should investigate immediately when oocyst are detected in raw water to establish if any circumstance exists to allow *Cryptosporidium* to enter water supplies. Investigations should include review of recent treatment plant operational data.

Water utilities should systematically asess and rank the potential risk of groundwater contamination by *Cryptosporidium* by application of a tripartite approach which assesses source, catchment and hydrogeological factors. Continued use should be made of existing national groundwater vulnerability maps and zoning schemes to assess risk of contamination wih *Cryptosporidium.*

The group recommends that water utilities carry out an assessment of risk from *Cryptosporidium* from each source and put in place a procedure for updating the review of risk assessment. Water treatment requirements and monitoring systems should be reviewed against the level of risk.

Water treatment works should be designed to handle the typical peak turbidity and colour loadings in source water.

Water treatment works should be operated at all times in a manner that minimises turbidity in final water; attention should be given to other parameters which reflect performance of chemical coagulation, that is coagulant metal concentration and colour

Coagulation/flocculation processes should be checked regularly to meet changing conditions of source water quality and other environmental factors.

Filters should be operated and maintained under optimum conditions with attention to the quality and depth of media abd to the operation of backwashing/airscouring systems.

For all sites at which *Cryptosporidium* might be a high risk, as determined by the risk assessment, monitoring should include continuous turbidity measurement on the outlet of each filter and on the final water using instuments capabl of detecting changes of less than 0.1 NTU.

During several of these outbreaks, oocysts were detected in the drinking water over a wide range of concentrations [Haas & Rose, 1995]. Haas & Rose proposed an action level of 10-30 oocysts in 100 l drinking water as a level above which outbreaks could occur. Craun *et al.* [1998] reviewed oocyst data from 12 outbreaks and found no association between observed oocyst concentration in drinking water and risk of illness. Examination of drinking water during outbreaks is usually too late to determine the concentrations that triggered the outbreak. This means that the water quality data are usually inadequate to determine if there is an association with illness. Gale *et al.* [2002] add the variability in oocyst concentration in drinking water as another factor that complicates establishing this association. To obtain 'historical' data on the occurrence of oocysts in drinking water, researchers have attempted to detect oocysts in ice [MacKenzie *et al*., 1994], in in-line filters [van Asperen *et al*., 1996] and in sediments of water storage tanks [Pozio *et al*., 1997]. The detected concentrations are probably an underestimation of the concentrations that led to the outbreak, although Haas *et al.,*[1999] showed for the Milwaukee outbreak that, with some assumptions, the measured concentration in drinking water was close to the predicted concentration on the basis of the attack rate, water consumption and dose-response relation. However, Hunter & Syed [2001]

argued that the size of the Milwaukee outbreak was actually much smaller, due to the use of an incorrect (low) background incidence. This would mean that the correlation between outbreak data and risk assessment data need to be revisited.

Low oocyst concentrations in drinking water have also been found in situations where no evidence for the occurrence an outbreak was present [LeChevallier *et al*., 1991; Karanis & Seitz, 1996; Rose *et al*., 1997; McClellan, 1998; Hunter, 2004]. Studies that have attempted to correlate the prevalence of parasites in drinking water to the prevalence of disease in the community receiving this water do not show a clear relation. This relation may be obscured by host immunity that could be triggered (and maintained) by low level exposure through drinking water and environmental sources. Most current detection methods do not allow the determination of infectivity of oocysts in water, which makes it difficult to determine the significance of low oocyst levels in drinking water. Given this uncertainty, detection of oocysts in treated water should always lead to the use of additional tests to confirm the presence of (potentially viable) *C. hominis* or *C. parvum* oocysts (molecular methods, using the 18S-rDNA gene and/or COWP-gene as targets [Xiao *et al.*, 2000; Amar *et al.,* 2004; Heijnen *et al.,* 2005]. Smith [2003] developed a method to genotype *Cryptosporidium* oocysts that were isolated from the slides used to detect *Cryptosporidium* with the conventional IFA method. If genotyping indicates the presence of *C. hominis* or C. *parvum* oocysts in relatively high numbers, this should lead to an epidemiological study to determine if significant waterborne transmission occurs and careful examination for the source(s) of the contamination and the installation of control measures (improved source protection and/or water treatment). Only when oocysts found in treated water are genotyped to determine whether they could potentially infect humans can the (in)significance of low numbers of oocysts in treated water be assessed. The molecular methods are sensitive enough for genotyping isolates from water, but unfortunately the current methods to determine whether oocysts are indeed infectious (mouse assay, cell culture) are not sensitive enough.The required sensitivity for the cell culture methods to work adequately for water quality monitoring is around 1 infective oocyst per litre.

3.3 SITE-SPECIFIC ASSESSMENT OF HAZARDOUS EVENTS.

3.3.1 Sanitary survey

A means to collect site-specific information on hazardous events is a sanitary survey. This is part of the Water Safety Plans' system assessment. It is the basis for effective strategies for prevention and control of hazards. Assessment of hazardous events includes understanding the characteristics of the drinking-water system, what hazards may arise and from which

sources, how these hazards create risks, and the efficacy of processes and practices that affect drinking-water quality. The complete system from catchment to tap should be described and analysed for events/conditions that could lead to contamination of the water supply. For a more detailed description of a sanitary survey, the reader is referred to the WHO background document on Water Safety Plans [Davison *et al.*, 2005]. Table 3 lists specific hazardous events that have led to *Cryptosporidium* outbreaks, as a basis for prioritising the hazardous events by risk of *Cryptosporidium* transmission. For microbiological catchment surveys of surface and groundwater supplies, the reader can find specific guidance in Medema *et al.* [2004].

Table 3. Examples of hazards leading to outbreaks of cryptosporidiosis [Adapted from Rose et al., 1997].

Deficiency	**Comment**
Catchment/source water	
Sources of high contamination were found near the treatment facility.	No mitigating barriers were in place to protect against introduction of oocysts into receiving waters (streams and groundwater) during periods of high runoff.
Sources of *Cryptosporidium* were unknown in the catchment prior to the outbreak event.	Knowledge of the sources of *Cryptosporidium* could have facilitated mitigation of the risk.
Natural events may have been instrumental in flushing areas of high oocyst concentrations into receiving waters.	Heavy rain can flush/carry oocysts into waters upstream of the treatment plant.
Water intake was localised in part of watershed vulnerable to peak events	Knowledge of the water system, contamination sources and transport hydrodynamics should be used in selection of abstraction site.
Treatment – surface water	
Monitoring equipment for filtration optimisation during periods of rapid change in source water.	Equipment was improperly installed, poorly maintained, turned off, ignored or temporarily inoperable.
Treatment plant personnel did not respond to faulty or inoperable monitoring equipment.	Deficiencies in the equipment were not compensated for by increasing the type and frequency of monitoring.
Filter backwash was returned to the head of the treatment process.	This process results in the possibility of concentrating cysts and oocysts, which may be put back into the system during a filtration breach.
Filtration processes were inadequate or altered.	During periods of high turbidity, altered or suboptimal filtration resulted in turbidity spikes and increased turbidity levels being noted in the finished water.
Filters were not adequately backwashed.	Slow start or filter-to-waste to prevent breakthrough.
Filtration was by-passed due to high demand.	By-passing of filtration without additional barriers may result in contaminated drinking water.
Absence of filtration	Filtration is essential to reduce the concentration of oocysts in source water to safe levels in most watersheds.
Groundwater	
Wells influenced by surface water	Rapid infiltration of surface water during rainstorms lead to rapid transport of micro-organisms from the surface water to the wells, leading

	to spikes in the abstracted water. Additional treatment or replacement of wells may be necessary.
Wells contaminated by seepage from sewer, septic tanks, sewage irrigation, manure	Adequate protection zones around wells where no contamination sources are present prevent this type of contamination.
Distribution	
Back siphonage	Absence of backflow prevention may result in back siphonage of toilet water.
Infiltration of sewage or manure into network	Distribution system integrity is impaired during construction and repair producing leaks that may allow infiltration of contaminants.
Open storage reservoirs	The probability of faecal contamination of open storage reservoirs is high.

3.3.2 Historical data

Historical data from monitoring of the source water, treatment processes, treated water and distributed water are also a potential source for site-specific identification of hazardous events. Data on the flow and turbidity of river water at the abstraction site can inform the water supplier of the frequency and magnitude of peak contamination events (esp. if the physicochemical data are supplemented with data on faecal indicator bacteria). An example can be found in the turbidity data of the off take of the water in Lake Burrangorang during the Sydney events in 1998 [Cox *et al.,* 2004]. An assessment of multiyear historical data on faecal indicators can identify the occurrence and magnitude of peak events (Figure 2) and help water suppliers to focus pathogen monitoring to these peak events.

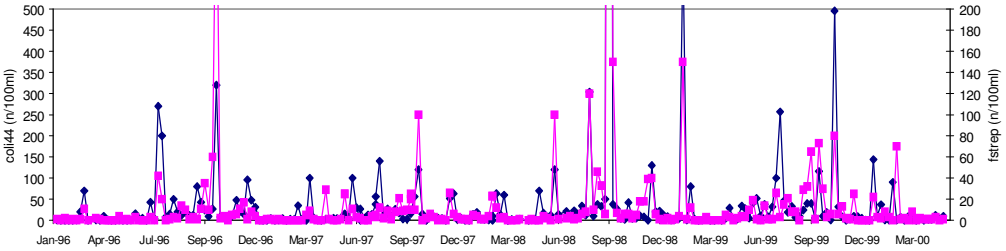


Figure 2. Multiyear data on thermotolerant coliforms (pink) and faecal streptococci (blue) in source water

Similarly, historical data on the performance of treatment processes, such as the data from turbidity monitoring or particle counting or the data on disinfectant dosing and residual, UV sensors etc., provide valuable information about the nominal performance and the occurrence of incidents of poor treatment performance (Figure 3).

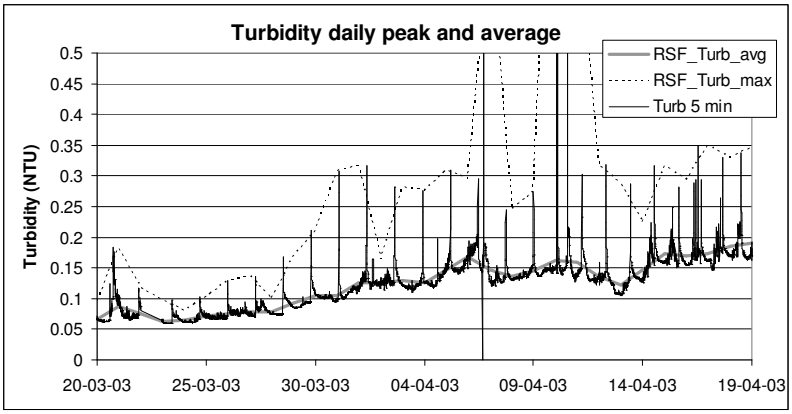


Figure 3. On-line turbidity data from the filtrate of a rapid sand filter, showing both gradual increase in effluent turbidity and the occurrence of turbidity spikes.

Also incident reports and operational logs can be used to determine the type of failures in treatment and their magnitude, frequency and duration, which is valuable for risk assessment [Westrell *et al.,* 2003].
The data from the monitoring programmes of the water in distribution networks (esp. *E. coli* and if available, enterococci) provide information about the occurrence of events of faecal contamination of water in distribution networks [Westrell *et al.,* 2003; Lieverloo *et al.,* 2006]. Also incident logs of larger contamination events provide information about hazardous events that have lead to contamination of the specific site.
Obviously, the physicochemical data and data on indicator organisms can only serve as indication of optimal or sub-optimal system performance. Whether these moments of suboptimal performance can lead to contamination of drinking water with pathogens and to what extend is less clear and usually assumptions are needed to deduce this information from the data.

In the UK, historical data on *Cryptosporidium* monitoring of treated water are available, as a result of the *Cryptosporidium* regulations that require daily monitoring of *Cryptosporidium* in at-risk water supplies [Lloyd & Drury, 2002]. Such data are of course particularly relevant for the identification of events in catchment, source or treatment that give rise to relatively high *Cryptosporidium* counts in finished water. Such an evaluation is currently being conducted with the regulatory data in the UK.

3.4 USE OF HAZARDOUS EVENTS IN QMRA

In the site specific assessment of hazardous events, hazards and hazardous situations are identified and prioritised using the sanitary survey, historical data on indicators or process parameters, or operational logs, or expert knowledge. These hazardous situations are significant information for risk assessment as they may comprise most of the health risk. Bartram *et al.* [2001] already identified that QMRA should not only be directed at the nominal performance of treatment systems, but also at the moments of poor source water quality and treatment performance.
After the individual hazardous events are catalogued, the events can be clustered into a risk scenario (i.e. heavy rainfall in the catchment leads to run-off of manure and sewer overflows resulting in high concentrations of *Cryptosporidium* in the source water). Simultaneously, the high turbidity of the source water renders disinfection less effective and may overload coagulation/filtration leading to breakthrough of the treatment. If the most relevant hazardous event can already be identified with the available knowledge, it may be effective to go back to the problem formulation and focus the risk assessment to this hazardous event.

4

Exposure assessment

This chapter describes how information can be obtained to determine the probability of exposure of humans to infective *Cryptosporidium* oocysts through drinking water. The exposure is determined by two factors:

- the concentration of viable and infective *Cryptosporidium* oocysts in drinking water at the point of consumption, which is usually very low;
- and the consumption of drinking water without further treatment (i.e. boiling) by the population.

In formula:

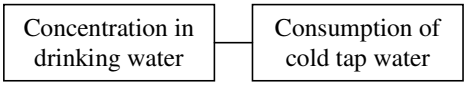
$$P_e = C_{dw} \times V \qquad (1)$$

Where:

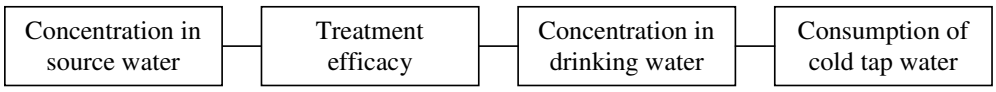
P_e = probability of exposure
C_{dw} = concentration in drinking water
V = volume of cold tap water consumed

Exposure can be defined as a single dose of one or more *Cryptosporidium* oocysts that a consumer ingests at a certain point in time, or the total amount of oocysts that constitute a set of exposures, i.e. over a day or a year. ?) As exposure to oocysts in drinking water is not

evenly distributed in time, it is important to assess not only the average exposure but also its distribution.
The next paragraphs describe how data and information can be collected to determine the concentration of *Cryptosporidium* in drinking water and the available information about consumption of cold tap water.
Two ways to assess the concentration of *Cryptosporidium* in drinking water are described. The first one is direct: monitoring of drinking water for the presence of *Cryptosporidium*.



The second one is indirect: monitoring of source water for the presence of *Cryptosporidium* and assessment of the efficacy of the water treatment processes in removing *Cryptosporidium* oocysts. Combining source water concentration and treatment efficacy yields an estimate of the concentration of *Cryptosporidium* in drinking water.



In current practice, both systems provide an estimate of the *Cryptosporidium* concentration in drinking water as it leaves the treatment works. Although post-treatment contamination is a significant source of waterborne outbreaks, the assessment of the contribution of this route is less well developed. A method is described to use the data on faecal indicator bacteria (*E. coli*) that are collected in distributed water.
But first, the methods for detecting *Cryptosporidium* in water are briefly described, as this knowledge is required to understand this discussion.

4.1 METHODS FOR DETECTION OF *CRYPTOSPORIDIUM* IN WATER

For a comprehensive overview of the detection methodologies, the reader is referred to the WHO Microbiology Review Document on protozoan parasites [Medema *et al*, 2001[b]] and the proceedings of the conference: *Cryptosporidium*: the analytical challenge [Smith & Thompson, 2001]. Here, the characteristics of the methods that are relevant for QMRA are briefly discussed.
The methods required for the detection of *Cryptosporidium* oocysts in water are different and more complex than the methods traditionally used in the water industry for the detection of bacteria. The overall procedure consists of several sequential stages, namely: sample collection and concentration, separation of (oo)cysts from contaminating debris and

detection/enumeration of (oo)cysts. Both large (100 – 1000 litres) and small (1-10 litres) volume sampling methods have been developed. Several different methods are used for sample concentration, filtration on flat membrane or cartridge filters, compressed foam filters, cross flow filtration, centrifugation and flocculation. After concentration, the oocysts have to be extracted and purified from the concentrate. This was originally done with density centrifugation, but is now commonly done with immunomagnetic separation. Some labs use flow cytometry with cell sorting (FACS) for purification. After the oocysts are purified, they are stained with fluorescent antibodies and detected under the epifluorescence microscope. The immunofluorescence assay is commonly used in environmental monitoring. This assay can be aided by FACS or laser scanning technology. Oocysts are identified by their fluorescence and morphology. Identification is facilitated by the use of the diaminophenylindole (DAPI) stain that stains the nucleus of the sporozoites. Characteristic morphology is verified by examining the oocysts under Differential Interference Contrast.
A recent development in detection methodology is harmonisation of the protocols used for environmental monitoring [EPA 1623 [1999], UKSCA [1999], Stanfield *et al.,* 2000, ISO [2004]]. Filtration is the most common method for the concentration, although different filter-types are used, and immunomagnetic separation is now the most commonly used technology for the separation of oocysts from sample debris.
The current methodology has several drawbacks that limit the applicability or interpretation of the results of *Cryptosporidium* monitoring, both in general and more specifically for the use of the data in QMRA. These drawbacks are outlined below.

4.1.1 Recovery efficiency

An important drawback of the current concentration methods is that many factors in the water matrix (suspended solids, algae) and also age/history of the (oo)cysts, can have significant effect on the recovery efficiency. Oocysts are lost in the subsequent concentration and purification steps, they are not eluted from the filter material or are not captured by the IMS. Suspended solids and divalent cations influence the binding of the IMS-antibodies to the oocysts and also ageing and oxidative treatment may strip the epitopes for antibody attachment from the oocyst wall [Vesey *et al*., 1993; Smith, 1996]. The actual concentration of oocysts in a water sample is thus (much) higher than the measured concentration. To estimate the actual concentration of oocysts, the oocyst counts need to be corrected for the recovery efficiency. This is complicated by the fact that the recovery efficiency can vary between samples.
The recovery efficiency can be measured by adding a known number of oocysts to the sample and process and analyse the sample. Seeding should be done in the water before the first concentration step, to approach the recovery efficiency of “natural” oocysts in the water sample.
Care should be given to the preparation of the oocyst suspension used for seeding, as purification procedures may alter the surface characteristics of oocysts [Reduker *et al*., 1985;

Brush *et al.,* 1998] that may affect the outcome of the recovery experiments. Also the age of the oocysts is a factor that may affect the recovery efficiency.
Ideally, the recovery efficiency is determined for every sample. Since this is laborious and expensive, recovery efficiency data are usually collected from a subset of samples. Warnecke *et al.* [2003] describe the use of pre-stained oocysts that can be discriminated from natural oocysts for seeding of every sample.
Teunis *et al.* [1997] regarded the recovery efficiency as a stochastic process; each oocyst has a certain Binomial probability of being recovered and this probability is not constant but follows a Beta-distribution. This allowed them to fit a Beta-Binomial distribution through recovery data to describe the variation in the recovery efficiency.

4.1.2 Viability/infectivity

Another drawback of the immunofluorescence detection assay is that is does not allow differentiation of viable from dead oocysts. DIC microscopy can be used to determine if the internal morphology is compromised as an indication of non-viability. DAPI (diaminophenylindole)-staining is used as support-stain that allows the assessment of the presence of sporozoites in the oocyst, again as mark of viability. Vital staining (PI [Campbell *et al.,* 1992] or Syto59 [Belosevic & Finch, 1997]) can be used in combination with the IFA test and gives an indication of cell membrane integrity. These dye exclusion assays provide some information about viability, but should be used with caution as they can (largely) overestimate viability of oocysts that have been exposed to stressors such as exposure to UV light [Clancy *et al.,* 1998].
Another assay to assess the viability of *Cryptosporidium* oocysts is cell culture. *Cryptosporidium* oocysts are able to infect cell-lines of human epithelial cells [Upton *et al.,* 1994]. The infection process and asexual reproduction of *Cryptosporidium* occur. Several cell lines are used; the lines from human enterocytes (HCT8) [Slifko *et al.,* 1997; 1999] and colon cells (CaCo2) [Rochelle *et al.,* 1997] and others. Immunofluorescence (foci method) or PCR are used to detect the presence of *Cryptosporidium* in the cell-culture. *Cryptosporidium hominis* and *C. parvum* differ in their ability to grow on different cell lines [Morgan-Ryan *et al.,* 2002]. Cell culture - PCR is currently used by one research group for the detection and quantification of oocysts in source water and drinking water [LeChevallier *et al.,* 2003], but is not used by others because the assay is considered too insensitive [Schets *et al.,* 2005].
If data on the proportion of non-viable oocysts are available from internal morphology or dye exclusion there are two ways to incorporate this information in the QMRA. The first is to use only the observed concentration of potentially viable *Cryptosporidium* oocysts in each sample, hence to discard oocysts that were counted as dead by these assays. The second is to combine the information from the (non)viability assays of the individual samples to determine the probability distribution of viability of oocysts in the water body that is assayed [Teunis *et al.,* 1997]. If count data are available from the cell-culture assay these can be used in QMRA directly [LeChevallier, 2004].

4.1.3 Specificity

The specificity of the immunofluorescence assay is based on the specificity of the monoclonal antibody-antigen reaction. Although this is highly specific, non-specific binding is observed in natural samples. Many of the particulates that react with the monoclonal antibody can be discriminated from oocysts by a trained observer, but occasionally particles (algae) occur in the sample that are very difficult to discriminate from oocysts [Rodgers *et al.,* 1995]. This may lead to false-positive results. In the Sydney events, this lack of specificity led to debate between researchers that argued that many of the observed oocyst-like particles could be algae versus others that considered them *Cryptosporidium* oocysts [Clancy, 2000; 2001].
The immunofluorescence method is also not specific to *Cryptosporidium* species and genotypes that are infectious to humans, also species that are infectious to animals are detected. Molecular techniques (PCR, genotyping) are rapidly evolving and some laboratories are now using these methods for environmental monitoring [LeChevallier *et al.,* 2003; Xiao *et al.,* 2001; 2004; Jiang *et al.,* 2005; Heijnen *et al.,* 2005]. The high specificity of genotyping methods is essential to understand the environmental transmission of *Cryptosporidium* [Latham *et al.,* 2003]. In the case of waterborne outbreaks, genotyping of the outbreak strain and strains found in the environment may elucidate the route of transmission and confirm or contradict the role of drinking water as route. Environmental samples are more likely to contain a cocktail of *Cryptosporidium* spp. than samples isolated from humans or animals. Genotyping can discriminate between the different *Cryptosporidium* species in environmental samples [Xiao *et al*., 2001] and also identify that multiple genotypes are involved in a waterborne outbreak [Xiao *et al.,* 2004]. Care is needed when applying PCR-RFLP analysis of one or several gene-loci, as comparison to microsatellite genotyping of stool samples showed that RFLP underestimates mixed infections [Chalmers *et al.,* 2005]. Genotyping methods can also be targeted to the question. In an outbreak investigation the question is whether the environmental isolates are identical to the outbreak strain and very specific methods such as microsatellite testing are applicable [Smith *et al.,* 2003]. In a QMRA the main question is how many oocysts of human pathogenic species are present, so typing to the subspecies level is not necessary but specific PCR(-RFLP) methods for *C. hominis*, *C. parvum* genotype 2 and *C. meleagridis* and maybe the *Cryptosporidium* species that are incidentally found in human cases.
PCR is not a quantitative assay. It can be made (semi) quantitative by assaying serial dilutions or by employing real-time PCR, where the formation of PCR-product in the reaction is monitored on-line and the onset of detection of PCR-product is related to the initial concentration of target DNA. A positive control is needed to test for inhibition of the PCR. The PCR gives information about presence of *Cryptosporidium* DNA in the sample. In combination with the concentration and IMS purification it can be argued that it is likely that this was no free DNA from the water sample but originating from the oocysts that were concentrated from the water sample. The PCR does not give information about viability or

infectivity of the oocysts. The PCR can be preceded by an assay that indicates viability (excystation [Filkorn *et al.,* 1994; Wagner-Wiening & Kimmig, 1995] or cell-culture [LeChevallier *et al.,* 2003], but these methods are either not specific for infective oocysts [Neumann *et al.,* 2000] or too insensitive [Schets *et al.,* 2005] for environmental samples.

4.2 MONITORING OF *CRYPTOSPORIDIUM* IN (UN)TREATED DRINKING WATER

Monitoring *Cryptosporidium* in untreated drinking water is the most direct way of assessing the concentration of *Cryptosporidium* in (finished) drinking water. Several studies have examined the presence of *Cryptosporidium* oocysts in treated drinking water (Table 4). Of the samples analysed (5 – 142), 3.0 – 40% of the samples contained oocysts in concentrations of 0.001 – 1.5 per litre. In the study of LeChevallier *et al.,* 1991, oocysts were found in 26.8% of 82 samples. All samples were taken from drinking water that was treated by filtration and the treatment systems had no recent history of sub-optimal operation. The highest concentrations were found in the study of Hsu *et al.* [1999] in Taiwan, China. The highest oocyst concentrations were found in systems using poor source water quality with high oocyst counts.

Table 4. Concentration of Cryptosporidium oocysts in drinking water during non-outbreak conditions (adapted from Rose et al., 1997 and Smith & Grimason, 2003).

Country	Filtra-tion	No. of samples	% positive	Concentration (n/l)	Reference
USA	Yes	82	26.8	0.001 – 0.48	LeChevallier *et al.,* 1991
USA	Yes	28	14.3	NA	Rose *et al.,* 1991
USA	No	6	33.3	0.001-0.017	Rose *et al.,* 1991
USA	Yes	262	13.4	0.00029 – 0.57	LeChevallier & Norton, 1995
Scotland, UK		142	40.1	0.007-0.72	Smith *et al.,* 1991
Germany	Yes	33	36.4	0.0013-0.21	Karanis *et al.,* 1996
Canada	No	423	3.6	-	Wallis *et al.*, 1996
Spain	?	9	33	0-0.02	In Smith & Grimason, 2003
Canada	No	42	5.0	0.002 – 0.005	Roach *et al.*, 1993
Germany		29	34		Wagner & Kimmig, 1992
South Africa				0.5	Kfir *et al.,* 1995
Taiwan, China	Yes	5	40	0.7-1.5	Hsu *et al.,* 1999
Japan	Yes	26	35	0.0015 – 0.008	Hashimoto *et al.,* 2002
Taiwan, China	Yes	31	39		In Smith & Grimason, 2003
Canada	Partly no	1760	3.5		In Smith & Grimason, 2003

UK	Yes?	209	37	0.007 – 1.36	In Smith & Grimason, 2003
Venezuela	?	11	90	0.004	In Smith & Grimason, 2003
UK	Mostly yes	201136	2.96	195183 samples <0.001 5608 samples 0.001 - 0.01 338 samples 0.01-0.1 7 samples >0.1 max. 0.491	Drury, 2004
USA	Yes	1690	24	NA	LeChevallier, 2004

In the UK, direct monitoring of drinking water is embedded in the drinking water regulation. Water supply systems that are at risk from *Cryptosporidium* are obliged to sample their treated water continuously. Samples of approx. 1000 litres water, taken over a period of at least 23 hours, are to be taken daily. The regulation requires water utilities to demonstrate that the drinking water is treated so as to ensure that the average concentration of *Cryptosporidium* is less than 1 oocyst per 10 litres of treated water. Although not put in place to assess exposure of consumers to *Cryptosporidium,* but rather as a treatment standard, the data that are collected under this regulation are applicable for risk assessment. In the UK regulatory monitoring of 2000 – June 2004, a total of 201136 finished water samples were analysed! In 195183 samples (84% of the water supply systems) no oocysts were detected. In 5953 samples, oocysts were detected in low concentrations (Table 4), with a maximum of 0.49 oocysts per litre. Since 2000, the percentage of positive samples declined from 8 to 1 %, partly due to improvements of treatment, and abandonment of risky supplies.

In unfiltered systems (with chlorination only and no UV), the *Cryptosporidium* concentration in drinking water can be estimated by monitoring source water. An example of such an approach is given by Haas & Eisenberg [2001], who used data from *Cryptosporidium* monitoring in the watersheds of New York City water supply. New York does not filter but treats the water from their pristine reservoirs with chlorination only. They had oocyst data from two watersheds. For each watershed, 292 data points were available, with many (85%) non-detects. The overall mean concentration in the two watersheds (with non-detects as zero) was 0.26 and 0.31 oocysts per 100 l.

LeChevallier [2004] studied the occurrence of *Cryptosporidium* in filtered drinking water with the cell-culture-PCR assay. In 1690 samples of 100 litres of treated water, 24 showed the presence of one or more infectious oocysts (1.4%). 153 samples were tested with the conventional method (EPA 1623) and 1 (0.7%) showed the presence of *Cryptosporidium.* He found no relation between detection of *Cryptosporidium* in treated water with the turbidity of the source water, type of clarification, plant capacity, backwash of filters and level of automation. Fewer positive samples were found in plants that used mixed filter media compared to sand or granular activated carbon alone. Small systems (serving <25000 people) were more often positive than large systems (31 vs. 20%) and small suburban systems were

more often positive than small urban systems (44 vs. 26%). Also plants of more than 75 years old were more often positive than younger plants (32 vs. 23%).

There are several major drawbacks associated with direct monitoring. The actual concentration of *Cryptosporidium* oocysts in drinking water is in most cases very low. In the absence of information on viability, infectivity and genotypes it is difficult to interpret monitoring data in terms of health risk. A very extensive monitoring programme is required to establish accurate information about the concentration. Spatial and temporal variation of microbial concentrations after treatment have been reported [Gale *et al.,* 2002; Hijnen *et al.,* 2004]. The likely result of an extensive monitoring programme with the current methodology will be a large number of non-detects and a low number of positive samples containing one or few oocysts. This is confirmed by most of the systems in the UK and also in the source water monitoring of New York City [Haas & Eisenberg, 2001] and Melbourne [Stevens, pers. comm.]. Although statistical distributions can be used to describe this type of data-set [Teunis & Havelaar., 1999; Evers & Groennou, 1998; Haas & Eisenberg, 2001], the level of uncertainty is high. As the cost of the methodology is high, large surveys are particularly expensive.
The methodological shortcomings, and especially the lack of specificity of the IFA-method for infectious *Cryptosporidium* oocysts, have led many researchers away from monitoring of finished water to focus on source water [Allen, 2000].

4.3 *CRYPTOSPORIDIUM* IN SOURCE WATER AND REMOVAL BY TREATMENT

In the indirect approach, the occurrence of *Cryptosporidium* oocysts in drinking water is calculated from their occurrence in source water and the removal efficacy of the treatment process. Source water monitoring for *Cryptosporidium* is applied in several countries, although usually in research rather than in routine monitoring.

4.3.1 *Cryptosporidium* in source water

Estimation based on watershed use

If no data are available on the presence of *Cryptosporidium* in a watershed, the average concentration of oocysts can be estimated very roughly from information on the level of faecal pollution of the watershed. This can be assessed by a sanitary survey [see Bartram & Howard, 2001] and available data on faecal indicator bacteria (*E. coli*, enterococci). Many studies have indicated that *Cryptosporidium* concentrations may peak during storm events or snowmelt, and that peak concentrations in source waters may be 10 – 100-fold higher than the concentration in non-event situations [Stewart *et al.,* 1997]. The potential occurrence of peak events should be taken into account in the sanitary survey and the estimation of the

Cryptosporidium levels on the basis of watershed use. In the classification below the average oocyst concentration is given. The maximum concentration in each of the classes will be 10-100-fold higher.

Very pristine

The watershed lies in an area without human settlements and activities like agriculture. Wildlife may be present but not in high densities. *E. coli* concentrations are typically below 1/100 ml. In such a watershed, *Cryptosporidium* oocysts may sporadically be present. Average concentration of oocysts can be estimated at 0.001 oocysts per litre.

Pristine

The watershed lies in an area with little and dispersed human settlement and small-scale agricultural activities. No direct input of human or livestock wastes is present. Wildlife is present. *E. coli* concentrations lie in the range of 1-10/100ml. *Cryptosporidium* is infrequently present. The average *Cryptosporidium* concentration can be estimated at 0.01 oocysts per litre.

Moderately polluted

The watershed lies in an area with villages and extensive agricultural activities (animal-based or foodcrops using manure or waste water sludge for fertilisation) are undertaken. Faecal wastes are collected and treated before discharged into the watershed. The intake of water for production of drinking water is not under the direct influence of wastewater discharges. The average *E. coli* count is 10-100/100ml *Cryptosporidium* is occasionally present. The average *Cryptosporidium* concentration can be estimated at 0.1 oocysts per litre.

Polluted

Small cities, villages and agricultural (animal- based or foodcrops using manure for fertilisation, feedlots) areas are present in the watershed. Wastewater is collected and treated before discharge in the watershed. Sewer overflows and agricultural run-off enter the watershed, but the water intake is not directly under the influence of these discharges. *E. coli* counts are typically around 100/100ml. *Cryptosporidium* is generally present. The average *Cryptosporidium* concentration can be estimated at 1 oocyst per litre.

Heavily polluted

Many and large urbanised areas and intensive agriculture (feedlots, large manure-storage facilities, intensive fertilisation with manure) are present in the watershed. Wastewater is generally collected and treated before discharge into the watershed. Average *E. coli* counts lies around 1000/100 ml. *Cryptosporidium* is generally present, in an estimated concentration of 10 oocysts per litre

Grossly polluted

Large urbanised areas and intensive agriculture are present in the watershed. Wastewater is generally not treated and/or manure is discharged into the watershed. The water intake may be under the direct influence of wastewater or manure discharges. The *E. coli* counts are usually above 1000/100 ml. *Cryptosporidium* is very generally present in average concentrations of 100 oocysts/litre and higher.

Cryptosporidium monitoring

Cryptosporidium has been found in very many surface waters worldwide. Many authors have reported the results of source water monitoring for *Cryptosporidium* oocysts [Madore *et al.,* 1987; Ongerth & Stibbs, 1987; Rose 1988; Stetzenbach *et al.,* 1988; Badenoch, 1990, 1995; Rose *et al.,* 1991; LeChevallier *et al.,* 1991; Smith *et al.,* 1991; Hansen & Ongerth, 1991; Roach *et al.,* 1993; Karanis *et al.,* 1996; Wallis *et al.,* 1996; Ong *et al.,* 1996; Medema, 1999; Dolesj *et al.,* 2000; Hsu *et al.,* 2001; Robertson & Gjerde, 2001; Medema *et al.,* 2001, 2003; Hashimoto *et al.,* 2002; Kistemann *et al.,* 2002; Tsushima *et al.,* 2003; Hormann *et al.,* 2004; Bastos *et al.,* 2004]. Although the interpretation of the results is hampered by the shortcomings of the detection and enumeration methods that have been used, these studies highlight several characteristics of the occurrence of *Cryptosporidium* in source waters. The most important of these is the variability of the oocyst concentrations. *Cryptosporidium* is found to be present in the majority of surface waters, but depending on the level of faecal pollution only few or almost all samples are found positive. The contamination level is related to the presence of the sources of faecal contamination, especially human sewage and run-off from agricultural land with cattle or sheep manure, in the watershed.

The concentration of oocysts at a surface water site varies considerably too. An overview of surface water surveys in the US showed that *Cryptosporidium* was found in 9.1 – 100% of the surface water samples in geometric mean concentrations of 0.003 – 1920 oocysts per litre [Rose *et al*., 1997]. Figure 4 shows the geometric mean and maximum concentration found in surface water from 38 studies in 16 countries, with a total number of 9354 samples. The mean percentage of positive samples found in these studies was 47% (5-95%: 5-100%). Most of the studies focused on source water for drinking water production; some included recreational water. Most of the studies reported oocyst concentrations without correction for the recovery efficiency of the detection method, some did correct the concentration. In the pristine sites, the geometric mean concentration was between 0.001 and 0.01 per litre, while maximum concentrations may rise to around 1 per litre. In heavily contaminated sites the geometric mean concentration is around 10-100 per litre, with maxima between 100 – 1000 per litre.

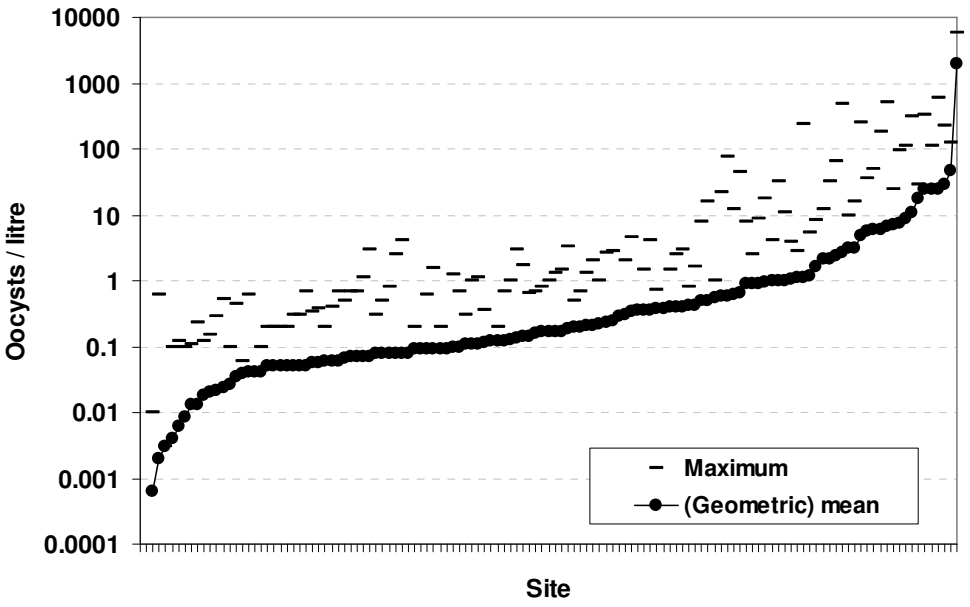


Figure 4. Cryptosporidium in surface water. Compilation of data from 9354 surface water samples from 16 countries (mostly developed countries). Surface waters ranged from pristine reservoirs in protected catchments to heavily contaminated rivers. Many studies reported geometric means, many reported arithmetic means and one study reported the median. The reported measures of general tendency are plotted in this figure.

In the US many data are available through the *Cryptosporidium* monitoring programs that were conducted under the Information Collection Rule (ICR) and ICR Supplementary Survey (ICRSS) [EPA, 2003]. Under the ICR, monthly data were collected over a period of 18 months at 338 filtered and 12 unfiltered water treatment plants, a total of approx. 6300 samples. Only 7% of these samples was found positive for *Cryptosporidium,* much lower than the avergae of the studies presented in figure 4. For filtered supplies the (arithmetic) mean concentration was 0.068 oocysts (all) per litre (90^{th} percentile was 0.194 / l). The mean concentration of oocysts showing specific internal structures was only 16% of the total oocyst concentration. For unfiltered supplies, the mean concentration was 0.002 per litre (90^{th} percentile 0.005 / l). In the ICRSS, 80 selected supplies (77 randomly selected filtered supplies and 3 selected unfiltered supplies), 24 samples were taken biweekly over a one year period (number of samples approx. 1900). In this survey 14% of the samples were positive, possibly due to the improved detection method (for the ICR this was EPA1622/1623, with a recovery efficiency of 43%, compared to 12% for the ICR-method). The mean concentration

was 0.06 oocysts per litere (90th percentile 0.1/l). Here, the mean conentration of oocysts with internal structures was 30% of the mean concentration of all oocysts. EPA has performed statistical data analysis on these data-sets [EPA, 2003].

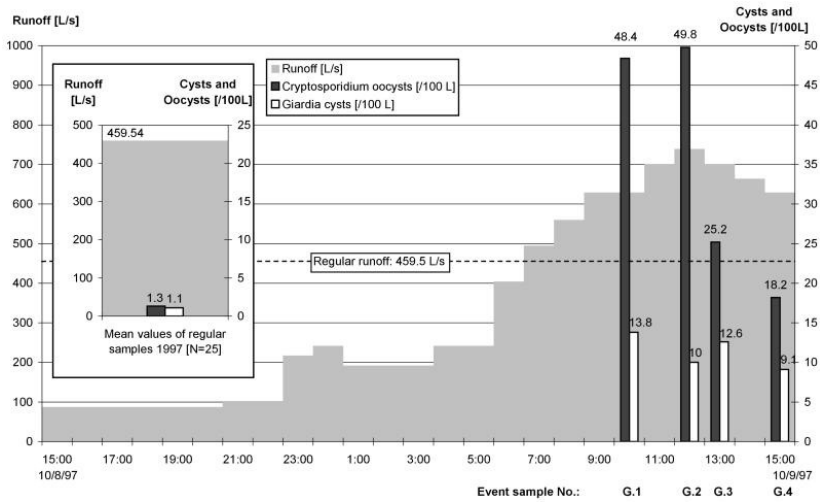


Figure 5. The increase of Cryptosporidium and Giardia concentration in a tributary to a drinking water reservoir following heavy rainfall (from Kistemann et al., 2002 (AEM)).

Despite the overall relation with the level of faecal contamination, the correlation between *Cryptosporidium* concentration and the concentration of faecal indicator bacteria is usually low [Atherholt *et al.,* 1998, Rose *et al.,* 1997, Medema, 1999]. Only spores of *Clostridium perfringens* are reported to correlate with *Cryptosporidium* concentrations in several studies [Atherholt *et al.,* 1998; Medema, 1999; Medema *et al.,* 2001].

Events such as rainstorms and snowmelt lead to run-off from agricultural lands and overflow of sewage systems. This gives rise to a sudden, sharp increase in the pollution of surface water with human and animal excreta. Several authors have shown that during these events the concentration of *Cryptosporidium* can be 10 – 100-fold higher than during non-event situations [Atherholt *et al.,* 1998; Ferguson *et al.,* 1996; Kistemann *et al.,* 2002 (see Figure 5)]. Peak precipitation or snowmelt events may not only lead to increased run-off but also in rapid travel of oocysts from source to drinking water off take or groundwater wells [Cox *et al.,* 2004; Medema & Stuyfzand, 2003]

The occurrence of short peak contamination events makes infrequent grab sampling less suitable for assessment of the concentration of *Cryptosporidium.* The likelihood that these peaks are missed is high, while these peaks represent periods of high risk. Atherholt *et al.* [1998] showed that the maximum concentrations found by monthly sampling was 7-fold lower than in a scheme were daily samples were taken for three weeks in each season.

As peak concentrations of *Cryptosporidium* are related to events such as heavy rainfall, several authors have used event sampling [Stewart *et al.,* 1997; Roser *et al.,* 2003]. So, rather than monitoring with a regular sampling program, (part of) the monitoring resources were dedicated to event sampling. This required knowledge of the catchment to identify sources of contamination and conditions that could lead to a peak event. Historical data on rainfall, snowmelt and river flow and turbidity may help to identify the conditions that lead to peak events and the "shape" of these events. As years differ, it is important to collect multiyear catchment data. The data can be used to tailor a monitoring program so peak events are included, but also to determine the frequency and magnitude of peak events.

4.3.2 Assessment of treatment efficacy

General

A comprehensive overview of the efficacy of treatment processes to remove/inactivate *Cryptosporidium* and other microbes can be found in the WHO review document on treatment efficiency by LeChevallier & Au [2004]. Also Smith & Grimason [2003] have reviewed the literature on water treatment processes against *Cryptosporidium* (and *Giardia*) and Hijnen *et al.* [2004] did the same for protozoa, viruses and bacteria, with a special emphasis on translation to full scale drinking water treatment.
In this document, only a brief description of the removal of *Cryptosporidium* oocysts by water treatment processes is given and generic removal efficiencies are derived from the published literature. For a more detailed description of the effect of treatment processes on *Cryptosporidium*, the reader is referred to the review documents mentioned above.

Chemical disinfection

Cryptosporidium is very resistant to chemical disinfection. The conventional disinfectants chlorine and chloramine have very little to no effect on the infectivity of *Cryptosporidium* [Korich *et al.,* 1990; Finch *et al.,* 1994; Chauret *et al.,* 1998]. Chlorine dioxide treatment may result in inactivation, but the required product of concentration and contact time (CT; disinfectant dose) is still high, especially at low temperatures [Finch *et al.,* 1997]. Ozone is the most potent oxidant, but the level of inactivation that can be achieved in drinking water practice is still very little at low temperatures [Peeters *et al.,* 1989; Korich *et al.,* 1990; Finch *et al.,* 1993; Gyurek *et al.,* 1999; Oppenheimer *et al.,* 2000]. Chemical disinfectants cannot be dosed to high concentrations because toxic by-products are formed by the reaction with compounds in the water, such as trihalomethanes by chlorine, nitrite (and NDMA?) by monochloramine, chlorite by chlorine dioxide and bromate by ozone.

UV

Although the older literature, employing *in vitro* viability assays, suggests that *Cryptosporidium* is very resistant to UV [Ransome *et al.,* 1993], Clancy *et al.,* 1998 showed that *Cryptosporidium* oocysts were highly susceptible to UV when assayed with mouse

infectivity tests. Several subsequent studies with mouse infectivity or cell culture assays have confirmed the high sensitivity of oocysts to UV, both from low and medium pressure lamps [Clancy *et al.,* 1998; 2000; 2003; Craik *et al.,* 2001; Shin *et al.,* 2001]. Identification of possible DNA repair genes in *C. parvum* showed that the oocysts contain all of the major genetic components of the nucleotide excision repair complex [Rochelle *et al.,* 2004]. Nevertheless, inactivation displayed by oocysts immediately after UV exposure or displayed by oocysts after UV exposure followed by various repair conditions were generally in the same order of magnitude, suggesting that oocysts will not regain infectivity after UV exposure.
These findings have resulted in an increased interest in UV treatment of the water industry and many surface water systems in the developed world are now evaluating the installation of UV for *Cryptosporidium* control.

Filtration

Filtration processes are important barriers for *Cryptosporidium* in water treatment. Before the discovery of the sensitivity of oocysts to UV, the attention of the water industry was focussed on oocyst removal by filtration processes and especially upgrading filter design and operations to optimise oocyst removal. Full scale conventional treatment with coagulation, floc removal and rapid granular filtration removes >2.3 logs [LeChevallier & Norton, 1992]. Other filtration processes, such as slow sand filtration and diatomaceous earth filtration give similar removal efficiencies for oocysts [LeChevallier & Au, 2004]. Membrane filtration can provide even higher removals of more than 4 logs, provided the integrity of the complete membrane system is well-maintained [Jacangelo *et al.,* 1995; Owen *et al.,* 1999].

Bank filtration

Bank filtration can be a significant barrier for *Cryptosporidium* [Medema *et al.,* 2000; Berger, 2002; Schijven *et al.,* 2003; Wang *et al.,* 2002; Gollnitz *et al.,* 2003; Plutzer *et al.,* 2007], but this strongly depends on the type of soil. Karstic soils have large fissures and cracks that allow rapid transport of oocysts without significant removal or inactivation. More fine grained soils (loam, loamy sand, gravel/sand) is effective in oocyst removal. A vulnerable situation for all bank filtration systems is a sharp rise in the river level (due to snowmelt or heavy rainfall). This may lead to peak events in source water in combination with rapid transport of water and oocysts in the soil [Medema & Stuyfzand, 2003; Dechesne & Soyeux, 2007].

Generic log-credits

Data on removal or inactivation of *Cryptosporidium* by full-scale water treatment processes are limited. Most of the data on treatment efficacy are collected in pilot systems or in laboratory experiments (jar-tests for coagulation, batch-tests for chemical disinfection and UV). Generally, challenge tests in the laboratory or in pilot plants tend to give more removal or inactivation than is seen in drinking water practice. This is due to differences in scale and hence the occurrence of short-circuits in full scale treatment processes [Smeets *et al.,* 2006],

to differences in the state of the micro-organisms in drinking water practice as compared to lab or pilot tests (attached to particles, more resistant state of micro-organisms in the environment). Full scale drinking water treatment is prone to variation [Medema, 1999; Hijnen *et al.,* 2000; 2004; Gale, Pitchers & Gray, 2002], due to variation in influent water quality, temperature, operations etc.. Data on the efficacy of treatment processes determined in lab and pilot tests should therefore be translated to full scale drinking water treatment with care.

In the US, the available literature is translated into "log-credits" for well-designed and operated treatment processes [EPA 1999, 2004; 2006]. In this approach, data from different studies are combined using mathematical [Clark *et al.,* 2003] or statistical [Qian *et al.,* 2004] approaches. The combination yields a single "log-credit" for the physical processes or a single dose-inactivation relationship for the disinfection processes. In the Netherlands, Hijnen *et al.,* [2004] reviewed the available literature on the removal of micro-organisms by UV, coagulation and slow sand filtration and translated this to log-credits for full scale water treatment processes.

These generic log-credits are helpful in a system assessment to determine the potential removal of *Cryptosporidium* by treatment processes. It must be borne in mind that generic log-credits are an approximation for removal by well-designed, maintained and operated treatment processes. Practical experience shows that poor design, maintenance and operation may greatly reduce the efficacy of water treatment. Generic log-credits are listed in Table 5 & Table 6 and can be regarded as 'default' values for well-designed, maintained and operated treatment processes. The generic log-credits can be higher in treatment systems that have installed special measures, such as intensive monitoring of turbidity or particle counts of individual filters as basis for the control of filter operation, intensive monitoring of the integrity of membrane filtration modules with regard to treatment monitoring, or detailed monitoring of the disinfectant dose over the contact chambers. The log-credits can also be lower when design is poor or the treatment processes are not well maintained and controlled (i.e. during peak events).

Table 5. Generic log-credits for removal of Cryptosporidium oocysts by well designed and operated filtration processes

Type of process	**Removal (^{10}logs)**	**Most important efficiency-determining parameters**
Pre-treatment		
Off-stream shallow storage reservoirs	0.5	Residence time, short-circuiting, resuspension of sediments

Dammed long deep stream reservoirs	2	Residence time, size, depth, short circuiting (esp. during temperature stratification), resuspension of sediments
Presedimentation basin with coagulation	0.5	Residence time, basin design, coagulant dose, temperature, pH
Microstrainers	0	Mesh size too wide for removal of pathogens
Filtration		
Rapid granular filtration	0.5	Filtration rate, recycling of backwash water
Rapid granular filtration with coagulation pre-treatment	2.5	Coagulant dose, pH, temperature, mixing, installation design, addition of polymers, recycling of backwash water
Slow sand filtration	2-4	Presence of "Schmutzdecke", filter depth, temperature, filtration rate
Diatomaceous earth filtration	3	Filtration rate, filter depth, pore size, precoat thickness, filter integrity
Membrane filtration	>4	System (membranes and connectors) integrity, membrane pore size
Coagulation/floc removal	1.6	Coagulant dose, pH, temperature, type of floc removal, installation design, addition of polymers, mixing
Soil passage		
Infiltration in aerobic sandy aquifer	3 log for the first 10 m, 1.5 additional log for every additional 10m	soil composition, residence time, travel distance, presence of sediment
Infiltration in anaerobic sandy aquifer	2 log for the first 10m, 0.5 additional log for every additional 10m	soil composition, pyrite content, pH, residence time, redox-state of the soil
Bank filtration in fractured bedrock, karst limestone etc	0	
Bank filtration in granular aquifers	2 log for the first 10 m, 1 additional log for every additional 10m	soil composition, residence time, high river flows

Table 6. Inactivation of Cryptosporidium by disinfectants (data taken from the draft Enhanced Surface Water Treatment Rule implementation guidance [Anon, 2004] and Hijnen et al. [2005])

	Disinfectant dose (Ct (mg min/l)/ fluence (mJ/cm^2) required for # log inactivation

Disinfectant	0.5	1	2	3
Chlorine	Not effective under practical conditions			
Monochloramine	Not effective under practical conditions[1]			
Chlorine dioxide				
1°C	305	610	1220	1830
10°C	138	277	553	830
20°C	58	116	232	347
Ozone				
1°C	12	23	46	69
10°C	4.9	9.9	20	30
20°C	2.0	3.9	7.8	12
UV	1	3	6	9

[1] Monochloramine has been reported to result in *Cryptosporidium* inactivation when following ozone disinfection. For inactivation CT tables of sequential disinfection with ozone and monochloramine, see Najm *et al.,* 2004.

Site-specific assessment of removal of oocysts by water treatment

The "log-credit approach" assigns a default-value for a treatment process, such as ozonation or slow sand filtration, while in practice such treatment processes differ in design, operation, feed water quality etc. Hence, there can be considerable differences from one water supply system to the other. McTigue *et al*. [1998], for example, reported an on-site survey in 100 conventional water treatment plants across the United States and demonstrated that the removal efficiency of particles greater than 2 μm ranged from 0.04 to 5.5 logs (median 2.8 logs). They also found a significant variation in the removal efficiencies of *Cryptosporidium.* Also within a site, pathogen removal may vary considerably, due to variations in feed water composition, temperature, operations, filter backwashing etc. [UKWIR, 1995; Gray *et al.,* 1999; Medema, 1999; Hijnen *et al.,* 2000]. *Cryptosporidium* removal may be significantly reduced during periods of suboptimal coagulation, at the end of a filter-run of just after backwashing if no filter-to-waste is applied [Huck *et al.,* 2001; Emelko *et al.,* 2003]. Variation is common to all water treatment systems and usually operations are dedicated to reduce the impact of variation in conditions on final water quality. For example, disinfection doses that are applied are higher when water temperatures are lower, coagulant aid is added when temperatures decrease, more coagulant is added when the turbidity of the feed water increases etc. So variation is normal and treatment processes and their operation are directed towards minimising the effect. On the other hand however, treatment operations may introduce variation. Turbidity or particle monitoring of treated water may show the filter backwash cycles reflected in turbidity/particle peaks in treated water (see Figure 3). In some cases the sources that lead to variation can even lead to treatment failure and the breakthrough of pathogens to treated water. For microbial water quality, even short-term variations and failures are critical, as short-term exposure to contaminated drinking water can give rise to microbial disease. Treatment failures have been associated with several outbreaks of cryptosporidiosis (Swindon, Milwaukee). In a theoretical exercise, Gale [2002] has shown that the impact of frequency of poor treatment performance ("bad days") is the main determinant of the health risk. In the same exercise he showed that if one filter in an

array of parallel filters in a treatment system operates poorly, this will greatly reduce the net removal of the whole system.

For site specific risk assessment and to incorporate the variability of the treatment processes, data on the removal of micro-organisms should be collected on-site, with sufficient frequency and duration to capture short- and long- (seasonal) variations.

Use of Cryptosporidium monitoring
An option to determine site-specific removal of *Cryptosporidium* oocysts is monitoring for *Cryptosporidium* before and after the treatment process. LeChevallier *et al.* [1991] collected data on the *Cryptosporidium* concentration before and after (coagulation)/filtration from 66 surface water treatment plants that used conventional treatment. The average reduction in *Cryptosporidium* counts was 2.38 ^{10}logs. Data from individual systems were too limited to assign local log-credits. In the UK, a substantial number of site-specific data are available on concentration of *Cryptosporidium* before and after treatment, but the treatment efficacy from these data has not been published.
Van Breemen [1998] and Medema [1999] published data on the concentration of *Cryptosporidium* in a river and after 3 subsequent pre-treatment storage reservoirs with an average residence time of 5 months. Teunis et al [1997; 1999] used these data to calculate an average removal efficacy of 1.94 logs.

Use of microbial surrogates
Cryptosporidium monitoring is expensive and suffers from the methodological shortcomings. Data are usually scarce. Several indigenous micro-organisms, that are ubiquitous in surface water and easy to analyse, have been used as surrogates for the removal of *Cryptosporidium* oocysts by conventional treatment. Of these, aerobic spores [Nieminski, 1997; Hall *et al.,* 2000; Facile *et al.,* 2000] and spores of sulphite-reducing clostridia/*Clostridium perfringens* [Payment & Franco, 1993, Deny *et al.,* 1992; Hijnen *et al.,* 1997; 2000; Medema, 1999; Montiel, 2002; Chung *et al.,* 2004] have been used most extensively. The average removal of Bacillus spores by filtration in 22 full scale systems corresponded with the removal of Cryptosporidium oocysts (both 1.6 – 1.8 log) [Nieminski, 1997]. Facile *et al.* [2000] determined the inactivation of lab-cultured *Bacillus subtilis* and environmental aerobic spores by ozone and compared these to published inactivation kinetics of *Cryptosporidium.* At 20-22°C, the CT required for 2 log inactivation of environmental aerobic spores was 1.72 $mg.min^{-1}.l^{-1}$ at pH 6.3, and 3.58 $mg.min^{-1}.l^{-1}$ at pH 8.2. This is comparable to the CT of 3.5 $mg.min^{-1}.l^{-1}$ that Finch *et al.* [1994] reported for *Cryptosporidium parvum* at 22°C, pH 6.9.
Other surrogates that have been used are algae [Akiba *et al.,* 2002], diatoms [Nobel *et al.,* 2002] and biological particles [Microscopic Particulate Analysis; Hancock *et al.,* 1996].
In pilot plant studies, Emelko & Huck [2004] showed that removal of seeded fluorescent polystyrene microspheres (4.6 µm, density 1.045 g/ml) correlated well with removal of seeded (inactivated) *C. parvum* oocysts by conventional and in-line filtration during a range of (suboptimal) treatment conditions. No full scale seeding trials have been reported.

Brown & Cornwell [2007] reviewed the data on aerobic spore removal and showed that they are a conservative surrogate for conventional physical removal processes, such as coagulation, clarification, filtration and bank filtration. Using the naturally occuring spores in source water of full-scale systems in the US, they were able to demonstrate up to 4 or 5 logs removal. The great value of such indicators is that they are much easier and cheaper to analyse, which allows water utilities to obtain solid data-sets on microbial removal of the full-scale system under the varying conditions of drinking water practice.

Data on the concentration of SSRC before and after treatment processes of surface water companies in the Netherlands were analysed to determine their elimination capacity [Hijnen *et al.,* 2000]. This survey has yielded a description of the removal of SSRC by coagulation/floc removal processes all under operational conditions (Figure 6).
The removal efficiency primarily depended on the type of floc removal process. Upward filtration is relatively effective process for primary floc removal, but in all the other systems, filtration is used as a secondary floc removal, rendering these systems overall more effective in floc removal. Within these process-types, the removal of spores by open sedimentation is relatively low, while flotation effectively removed spores.

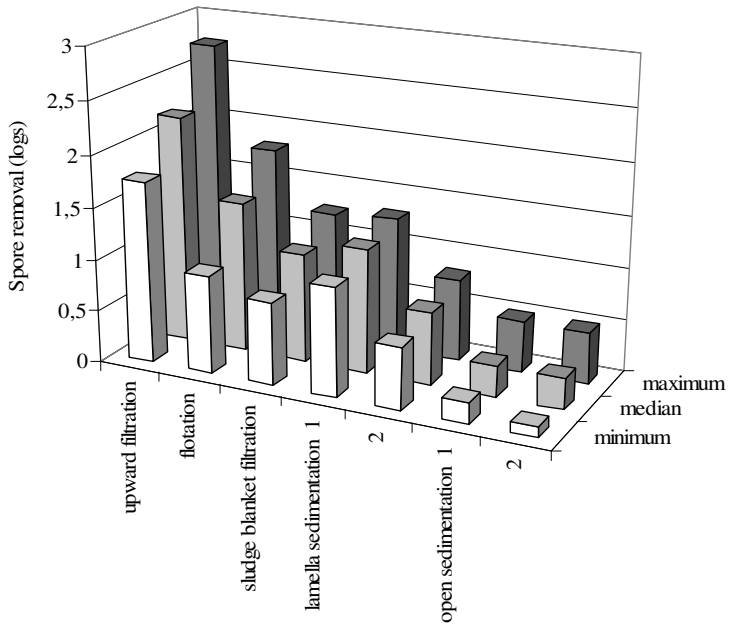


Figure 6. Removal of spores of sulphite-reducing clostridia by various types of coagulation filtration processes.

As SSRC concentrations after the final treatment steps were low, the method was scaled up to be able to analyse 100-500 litres of water [Hijnen *et al.,* 2000]. This increased sensitivity has allowed the assessment the efficiency of full scale treatment systems under operational conditions (Figure 7). With the conventional method, >2.2 logs removal could be demonstrated, while the high sensitivity method allowed the demonstration of 3.5-4.2 logs removal.

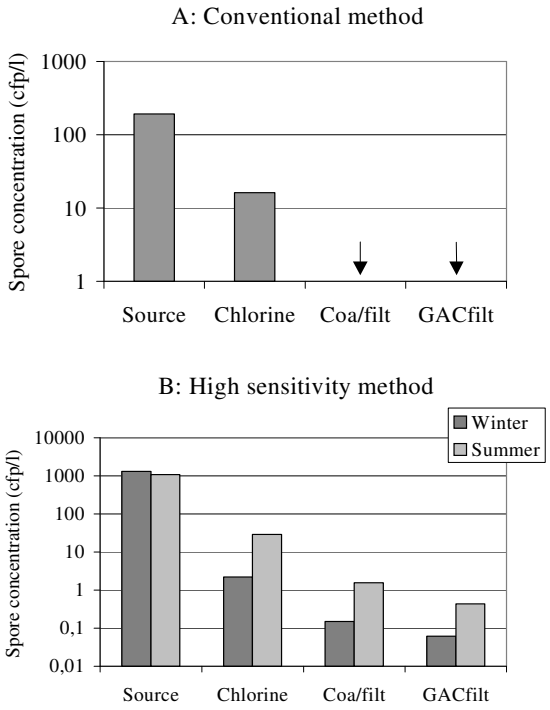


Figure 7. Elimination of spores of sulphite-reducing clostridia under full-scale conditions. A: conventional methods; the efficiency of the latter treatment processes cannot be demonstrated. B: high sensitivity method; the efficiency of all treatment processes and the overall treatment can be demonstrated.

None of the surrogates is perfect and the validity of the use of surrogates to get a quantitative estimate of the removal of *Cryptosporidium* by water treatment processes should be demonstrated in comparative studies. Nonetheless, the relative ease of data collection of full scale systems is of great value to determine treatment performance, especially to determine the removal under suboptimal process conditions.

In the case of microbial count data, data analysis can be done according to the method described by Teunis *et al.* [1999b.]. They have described how data before and after a treatment process can be combined. In drinking water practice, samples before and after treatment are usually collected on the same day and these samples could be regarded as paired samples from which the removal efficacy of each day could be calculated. It should be borne in mind that this approach may be valid for treatment processes where the in- and outlet sample reflect the same body of water with suspended micro-organisms, such as in disinfection processes. In several treatment processes, samples taken on the same day are not samples of the same water body before and after treatment and cannot be regarded as paired samples. An illustration is the dataset on *Cryptosporidium* before and after reservoir storage with an average residence time of 5 months [Medema, 1999]. Even if sampling does analyse the same water body before and after treatment, by taking the average water residence or contact time in the treatment into account, micro-organisms may not be transported through the treatment in the same manner as water is transported. An example is slow sand filtration where *Cryptosporidium* oocysts can be retained on the filter and may be dislodged over time or during increased flows. For such processes, the log-credits should be derived from the combined inlet and combined outlet samples (average concentration in/average concentration out). The variation in the concentration of micro-organisms can give an indication of the variability of the removal.

Use of turbidity and particle counts

Pathogen measurements are not very suitable to collect data on the variability of treatment efficacy in full scale systems, because they are too laborious and have too much inherent variability. Also, many pathogens may not be detectable in source water when there is no constant fecal source. This is especially true for waters in natural areas or waters affected by smaller sewage systems or few individual treatment systems, (e.g., septic tanks.) To a lesser extend, this is also true for all current microbial parameters as the sampling frequency is still low (usually once a week, sometimes once a day) and covers only a small proportion of the time treatment processes are in operation. Non-microbial surrogates that can be monitored on-line are used for process control. Water suppliers usually have multi-year data of process or water quality parameters such as turbidity, disinfectant residuals, coagulant dose etc.

Several process parameters can be used to assess the efficacy (and its variation) of full scale filtration systems in (near) real time. Examples are counting of small-size particles and turbidity. Nieminski & Ongerth [1995] evaluated the removal of *Cryptosporidium* oocysts (and *Giardia* cysts) at pilot and full-scale conventional (and direct) filtration plants. Dual-media filters with anthracite and sand were used. *Cryptosporidium* oocysts were effectively removed when the plants produced filtered water of low turbidity (0.1 - 0.2 NTU). Under optimal coagulation conditions, the average removal of *Cryptosporidium* was 2.3 logs. A correlation was found between removal of *Cryptosporidium* and particle (4–7 μm) removal (R^2 =0.79) and removal of turbidity (R^2=0.64).

Gale & Stanfield [2000] have used the data on particle removal from 67 different conventional treatment plants to determine the variability of *Cryptosporidium* removal. This is not the same as the variation of one conventional treatment plant over time, but can serve as a first estimate of the variance in treatment performance.
LeChevallier & Au [2004] discuss an important limitation of turbidity and particle counts. They refer to McTigue *et al.* (1998), who used pilot filtration plant experiments and the level of *Cryptosporidium* in feed water was varied from 26 to 4610 oocysts/l. Monitoring of the plant effluent showed a consistent removal of approximately 4 logs. Turbidity and particle count data, which were limited because of relatively low levels in source water, showed an apparent removal of 1.0–1.6 logs (Table 7). Hence, removal of oocysts is considerably better than indicated by the surrogates. The value of particle or turbidity removal by filtration

Table 7. Impact of source water concentration on apparent treatment performance (from McTigue et al. [1998]).

Oocysts/l			Turbidity (NTU)			Particles > 3 µm/ml		
Raw	Effluent	Log removal	Raw	Effluent	Log removal	Raw	Effluent	Log removal
26	0.0017	4.2	2.5	0.07	1.6	7000	350	1.3
688	0.0410	4.2	2.0	0.07	1.5	7700	530	1.2
4610	0.2140	4.3	1.3	0.07	1.3	4700	480	1.0

NTU = nephelometric turbidity unit

to describe the log-removal of *Cryptosporidium* oocysts by conventional filtration processes is therefore limited.
Turbidity and particle counts have more value in assessing on-line monitoring process performance and can indicate changes in the removal of particles (and hence micro-organisms) due to changes in feed water or operations. In the UKWIR study [200?] and in Edwards [2000] several examples of the value of particles are given: to identify poor performance of individual filters or the slow decrease of the performance of a filter over time, to monitor the performance increase during filter-to-waste or slow-start of filters after backwashing, to determine the performance decrease towards the end of the filter-run and to identify the effect of flow surges. Employing particle counts to monitor the removal of pathogens by ultrafiltration in drinking water practice at Heemskerk, the Netherlands [Willemsen-Zwaagstra *et al.,* 1997], particle removal with newly installed membranes indicated 5log removal. After months of operation, the particle counts after the UF gradually increased, which was attributed to loss of integrity of individual membrane filter units. After replacement of these units, the particle removal was 5 logs again [Kruithof *et al.,* 2001].
Hence, turbidity and particle counts are very useful in monitoring filtration systems on-line to detect breakthrough of particles through filtration as early as possible. They are therefore a valuable tool for the treatment operator to optimise and maintain filtration performance, and to indicate when corrective actions are required because filtration performance is compromised.

Use of treatment models

Several treatment models have been used; Haas *et al*. [2000] produced a model for removal of *Cryptosporidium* by coagulation, flocculation, and sedimentation, using coagulant concentration, polymer concentration and process pH to describe the factors which log removal of oocysts was dependent upon. The model had an excellent fit to the data (R^2 of 0.94), although incorporation of more data-sets reduced the fit. Disinfection efficacy has been modelled by several models, all using disinfectant concentration and contact time to describe inactivation efficacy. The disinfection models are simple first-order kinetics ($N/N0=e^{-kCt}$)[1] Chick's model) or have more parameters to improve the fit on disinfection data [Chick-Watson: $N/N0=e^{-kCnt}$). For ozone disinfection of *Cryptosporidium*, Finch *et al*. [1993] used the Hom model ($N/N0=e^{-kCntm}$).

Teunis *et al.,* [1999] and Havelaar *et al.,* [2000] have used the Hom model from Finch *et al.* in combination with an ozone demand and decay model and a contact time distribution model (inverse Gaussian) to calculate the inactivation of *Cryptosporidium* oocysts, assuming a constant ozone dose.

In the monograph on micro-organism removal by water treatment, LeChevallier & Au [2004] describe the use a model for removal of particles by filtration to describe *Cryptosporidium* removal. The model uses a combination of transport of particles from the suspension to the filter grains and the attachment of particles to the filter grains. The probability that a particle touches a filter grain is governed by intervention, sedimentation and diffusion and the probability that this particle attaches to the grain is governed by physicochemical (electrostatic) processes. The model appeared to predict removal efficiencies of *Cryptosporidium* oocysts by gravity filters in the same order of magnitude as experimental data [LeChevallier & Au, 2004].

Use of failure reports

Water suppliers may also have failure/incident reports that can be used to determine frequency, magnitude and impact of treatment failures. These data and reports can be used to determine the removal of *Cryptosporidium* oocysts, both the average removal and the variation over time. Westrell *et al.* [2003] describe the use of failure reports in treatment (and distribution, see 4.3.1) to estimate the health risk for the consumers. The type of treatment failure and its duration were documented in the failure reports. For the water supply of the city of Gothenburg, they identified an annual failure frequency in filter operation of 15 times with an average duration of 5 hours, chlorination failure frequency was once every two years for 0.4 hours and coagulation failure frequency of 1.5 times per year for 0.6 hours. They estimated the impact of these failures on the concentration of pathogens in treated water and the resulting exposure of the consumers (Figure 8). In this system, they estimated that the

[1] N and N_0: concentration of micro-organism at time t and time 0, respectively; k, n, m = empirical constants, C= dissinfectant concentration; t = contact time

nominal operation of the treatment resulted in a higher annual probability of infection than the infrequent and relatively short failures.

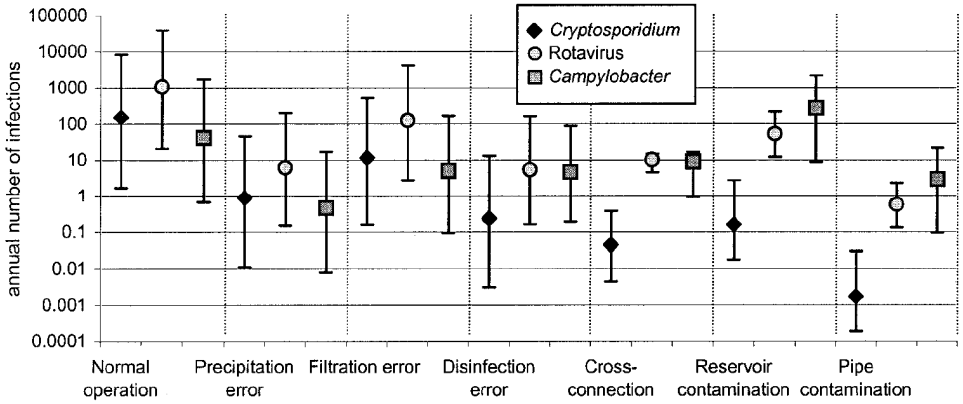


Figure 8. Median (with 95% error bars) annual infections per 250,000 inhabitants resulting from normal operation, failures in treatment and in distribution in the water supply system of Gothenburg (from Westrell et al., 2003).

Synergism between processes

Synergism (two processes produce more than the sum of the single processes) between processes has been described for disinfection processes [Oppenheimer *et al.,* 2000; Driedger *et al*., 2000; Biswas *et al*, 2003]. However, *Cryptosporidium* is extremely resistant to chlorine and chloramines and even in combination the inactivation of *Cryptosporidium* under drinking water treatment conditions is limited. The combination of ozone and chlorine dioxide and ozone and chlorine have also been shown to be synergistic, the latter only at low pH (around 6.0).

An important observation from the data on removal of *Clostridium* spores by a chain of treatment processes is that the level of variation in the efficiency of the overall treatment is not as large as would be expected from the adding the removal data from the individual processes (Table 8). This indicates that the variation in one treatment step counteracts the variation from the previous treatment step.

Table 8. Mean and coefficient of variation of the removal of spores by subsequent treatment steps. Note that the variation (coefficient) of overall treatment is smaller than the variation of the individual treatment steps

Treatment process	**Mean DE**	**Coefficient of variation**
Chlorine	1.53	15.4

COA/filtration	1.54	37.8
GAC-filtration	0.45	143
Overall	3.39	14.3

This means that multiple barriers in treatment not only increase the overall removal capacity, but also reduce the variation in the overall treatment efficiency and thus the risk of breakthrough of pathogens to drinking water. This interaction should be incorporated in the description of treatment efficiency, using statistical methods. There is a specific WHO document in preparation that describes the effect of treatment fluctuation and the interaction between different treatment processes on microbial removal.

4.3.3 Post-treatment contamination

Post-treatment contamination is a significant hazard. When the water in the distribution system or in storage containers is contaminated, no barriers are in place to prevent ingestion of infectious *Cryptosporidium* oocysts. Post-treatment contamination may occur through infiltration of contaminants in the distribution system through leaks (during surges), in open distribution reservoirs or other open connections and during construction and repair. Cross-connections and back-siphonage may draw water from toilets or sewers into the network. Storage tanks used in houses (i.e. in standpipe systems) may also become contaminated. Several outbreaks of cryptosporidiosis have been caused by post-treatment contamination [Hrudey & Hrudey, 2004].
Once introduced into the distribution network, *Cryptosporidium* may settle in the sediment or biofilm in the network. Settled oocyst may survive for prolonged periods in the sediment or biofilm, although no information is available about the residual infectivity of oocysts in biofilms. Settled oocysts may be resuspended in the water phase during high water flow. After the initial findings of *Cryptosporidium*-like particles in the distribution network of Sydney, the network was flushed to remove the contamination, but the number of oocysts found in samples after flushing was higher than before flushing. It was suggested that this was due to oocysts that were present in the sediment [McClellan, 1998]. Information about the presence and infectivity of *Cryptosporidium* oocysts in distribution system sediments is lacking.

Except for the reported outbreaks, no data are available about the presence of *Cryptosporidium* in distributed water. An indirect assessment of the frequency and magnitude of faecal contamination of distributed water can be done with the data from the monitoring programs for *E. coli*. Water suppliers have many years of data on *E. coli* samples from the distribution network available from statutory monitoring programs. In addition, water suppliers have incident reports, describing the occurrence of contamination incidents and the response to these incidents. Both types of information are generally the only site-specific information available and can be used to estimate the frequency of faecal

contamination and give an idea of the magnitude of these contaminations. In studies in the UK and the Netherlands, overall 0 – 0.2% of the samples in the distribution network were positive for *E. coli*, although some individual distribution networks could have higher percentages of samples in which thermotolerant coliforms were detected [van der Kooij *et al.,* 2003; Lieverloo *et al.,* 2006]. Westrell *et al.* [2003] collected distribution incident reports from the water supply of Gothenburg over a 20 year period and used either the flow ratio of sewage vs. drinking water during cross connections with pressurised sewerage systems or the coliform data during contamination incidents of unknown origin in reservoirs or the periphery of the network. The coliform data in water were compared to those in sewage to calculate the degree of contamination and translated these to *Cryptosporidium* concentrations. The calculated risk of infection was $< 10^{-5}$ per person per year (pppy), and was considerably lower than the 10^{-3} pppy that they calculated from normal treated water (figure 4.5). Lieverloo *et al.* [2006] also investigated incident reports of water companies. Eight water suppliers in the Netherlands reported 9 faecal incidents (repeated detection of *E. coli* in distributed water) over a period of 5 years, although this was considered to be a lower estimate as not all incidents were documented. Data on the duration and magnitude of these incidents were collected. Under the (worst case) assumption that the incidents were due to contamination with fresh sewage, they used the concentration of thermotolerant coliforms during the incidents and the average concentration in sewage to calculate the contamination level. Using concentration data of *Cryptosporidium* in sewage, they estimated the concentration of *Cryptosporidium* in tap water during these incidents. They calculated a risk of infection due to *Cryptosporidium* of up to 10^{-5} pppy during incidents.

These assessments should be regarded as a rough approximation. Monitoring is infrequent and the probability that contamination events are missed is high. Water transport patterns in larger distribution systems are complex and variable, so it is not easy to deduce a contamination level from infrequent grab samples.

A faecal contamination event that has led to the presence of *E. coli* in distributed water may have come from various sources, where *Cryptosporidium* may have or may not have been present. If the assumption is made that the origin of the faecal contamination is the ingress of fresh domestic wastewater, the available literature data on *Cryptosporidium* concentrations in sewerage can be used to make a rough worst case estimate of the concentration of *Cryptosporidium* in the contaminated drinking water.

In a theoretical exercise, Pouillot *et al.,* [2004] calculated the probability of infection from different concentrations of *Cryptosporidium* in a distribution reservoir. The concentration of *Cryptosporidium* in reservoir water was considered to be Poisson distributed (random) and combined the concentration data with the data from a French water consumption survey [Beaudeau *et al.,* 2003] to calculate the probability of infection. The relation they found between *Cryptosporidium* concentration and probability of infection (with confidence range) is plotted in Figure 9.

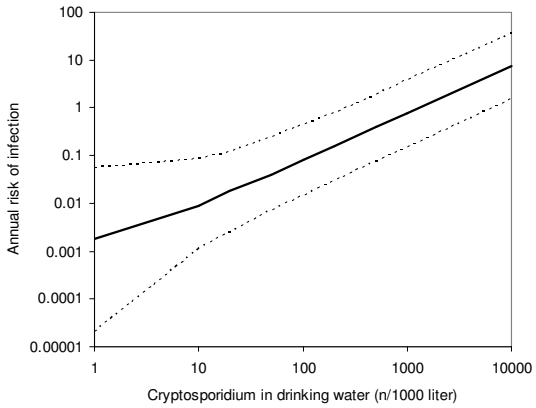


Figure 9. Probability of infection associated with Cryptosporidium concentrations in tap water (adapted from Pouillot et al., 2004).

Obvious source of uncertainty is the assumption that the source of contamination is domestic wastewater and the presence and concentration of *Cryptosporidium* in domestic wastewater, which would depend on the size of the population served by the collection system. Other sources may have been responsible for the contamination. Wildlife sources will yield a lower probability of contamination with *Cryptosporidium,* given the low prevalence that is reported in wildlife and the absence of genotypes that can cause illness in humans (see Chapter 2). If the source is fresh calf manure, the probability of *Cryptosporidium* presence and the expected concentration of oocysts in the contaminated water are higher than with domestic wastewater (see Chapter 2).
If the distributed water is chlorinated, *E. coli* could be less suitable for translation to *Cryptosporidium* occurrence, because *E.*coli is much more sensitive to chlorine than *Cryptosporidium.*

Overall, the information to incorporate contamination of water in the distribution in QMRA is limited. The approach used by Westrell *et al.* [2003] and van Lieverloo *et al.* [2006] can be used as a first assessment, but the main uncertainty is the concentration of pathogens in distributed water during incidents.

A recent case control study on sporadic cryptosporidiosis in the UK reported an association between gastro-intestinal illness and the loss of water pressure in the distribution network [Hunter *et al.,* 2005]. Twenty eight of 423 controls reported diarrhoea in the two weeks before the questionnaire. Analysis of the risk factors showed a strong association with the loss of water pressure at the household tap. Most of these pressure-losses were associated with reported events in the distribution network, such as a burst of water mains. They

suggest that failures in the distribution network could have a significant contribution (around 15%) to the overall rate of gastro-enteritis in the population.

4.4 CONSUMPTION OF DRINKING WATER

The other component of exposure assessment is the volume of water consumed by the population. Not only the average volume of water consumed is important, also the person-to-person variation in consumption behaviour and especially consumption behaviour of risk groups (in terms of sensitivity to infection or high level of consumption) is relevant.
When assessing the consumption of drinking water, only tap water that receives no additional (heat) treatment that will inactivate or remove *Cryptosporidium* should be considered. Heat treatment (i.e. for coffee, tea or cooking) at temperatures of 60°C and higher rapidly inactivates oocysts [Harp *et al*., 1996]. After 15s at 72°C, no infectivity could be observed in 177 doses of 10^5 oocysts in neonatal mice, which (given the ID_{50} of oocysts in neonatal mice) may be considered equivalent to > 5 log reduction. Also other means of point-of-use treatment eliminate oocysts. Membrane (micro- or ultra-) filtration units can be absolute barriers against oocysts [Jacangelo *et al*., 1995]. Exposure of tap water to UV or sunlight inactivates oocysts. A significant inactivation occurs at low UV-doses (>3 logs at 19 mJ/cm^2; Bukhari *et al*., 1999].
In addition to drinking, tap water (or in containers in the case of non-piped supplies) is used for other purposes (ice, beverage and food preparation, personal hygiene, laundry etc.) that result in ingestion of drinking water.

Data on the volume of tap water consumed are available for several high-income countries. In previous risk-assessment studies consumption data from several studies were used. In the first risk assessments, an average consumption of 2 litres/day was used [Regli *et al*., 1991]. Roseberry & Burmaster (1992] performed a survey of drinking water consumption in the USA. This is, however, the consumption of both untreated and boiled or heated drinking water. The survey did provide data on the variation of tap water consumption within a US population. These data were used in several risk assessment studies [Medema *et al.,* 1995; Haas *et al.,* 1999; Haas & Eisenberg, 2001; Westrell *et al.,* 2003]. In Canada, similar tap water consumption is reported [Haas *et al*., 1999].
Teunis *et al.,* [1997, 1999] used consumption data from a food intake population survey in the Netherlands, the median intake of unboiled tap water was 0.152 litres per day, much lower than the intake data of total tap water intake from North America.

The survey of Roseberry and Burmaster also provided age-specific distributions of tap water consumption, with a tendency towards increased consumption with age; the elderly tend to consume more tap water. A survey by Ershow *et al*. [1991] indicated that in the proportion of persons consuming > 2 litres of tap water was relatively high in pregnant women (15%). The elderly and pregnant women are sensitive groups with an increased risk of severity of gastro-intestinal illness and mortality due to several waterborne pathogens [Haas *et al*., 1999].

There are however no specific data that suggest that these groups are also at risk of more severe cryptosporidiosis.

Studies from other areas are available but have not been used in risk assessment studies. The information on consumption of tap water is collected in different types of studies, such as in epidemiological studies of waterborne outbreaks; in general food intake surveys and in specific tap water intake studies. Different study designs are used. The impact of study design on the outcome is currently reviewed [Mons *et al.* 2007]. They re-analysed the data from several consumption studies and recommended the use of country-specific data where available. If no country-specific data are available, they suggested to use the relatively conservative (high consumption) dataset that was collected by Robertson *et al.* [2000] in Australia (**Error! Reference source not found.**), in risk assessment studies.

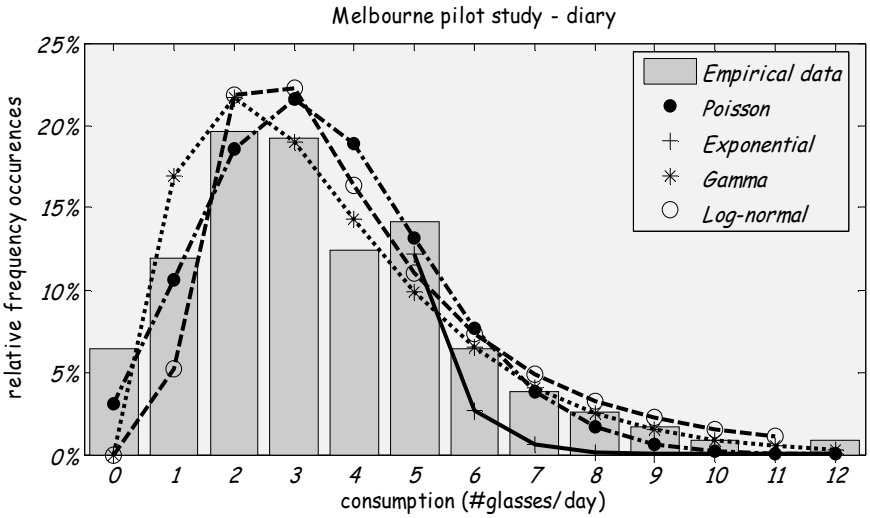


Figure 10 Statistical probability distributions for discrete momentous tap water consumption Melbourne pilot study – (diary study, Robertson et al., 2000).

5

Effect assessment: dose-response relation

5.1 HOST CHARACTERIZATION

The probability that exposure to micro-organisms will result in infection or illness depends on both properties of the host and of the pathogens. Different strains of the organisms may differ in their ability to infect or cause illness, by their genetic make-up, but there may also be effects of the environment (time since they were excreted from the previous host, susceptibility to adverse stimuli, see chapter 4 on pathogen characterization). Different human hosts may also differ in their susceptibility to the pathogens, because they are (genetically) predisposed, or because of previous experience (acquired resistance), or dependent on the specific circumstances of exposure, for instance the vehicle (water or some buffered suspension) or behaviour (recreational exposure, associated with immunosuppressive UV levels). Ultimately, health effects result from the interaction between pathogen and host, and it is the combination of pathogen and host factors that determines the actual risk.

Age is usually considered an important factor in the susceptibility to pathogenic micro-organisms. In humans, cryptosporidiosis is prevalent among small children [Wiedermann *et*

al. 1985]. While there may also be an association with unhygienic behaviour (causing increased exposure), the immature status of the intestinal tract, as well as mucosal immunity, may be mainly responsible for this increased susceptibility.
Human feeding studies have shown that adults are susceptible as well [DuPont *et al*. 1995, Okhuysen *et al*. 1998, 1999, Chappell *et al*. 1996, 1999]. While there is as yet no information on potential heterogeneity in innate susceptibility among humans, a protective effect associated with an immune response has been demonstrated [Chappell *et al*. 1999]. Other evidence for such an association comes from cryptosporidiosis in immunocompromised subjects, where illness is much more persistent and severe than in normal immunocompetent subjects. This is especially the case in HIV patients or patients receiving cancer chemotherapy who have low CD4 T-cell concentrations. In these patients, chronic cryptosporidiosis is reversed when CD4 counts can be elevated.

5.2 HEALTH EFFECTS

Most reports on cryptosporidiosis are based on outbreaks, travellers' diarrhoea, and *Cryptosporidium*-infected HIV-patients. The incubation period for illness symptoms is approximately 7 days (range 1-14 days) and illness is usually self-limiting, with a mean duration of 6-9 days [Dupont *et al.,* 1995; Palmer & Biffin, 1990], although longer times (mean duration 19-22 days, maximum up to 100-120 days) were reported in a recent survey in Australia [Robertson *et al.,* 2002]. Relapses are common; reports indicate 1-5 additional episodes in 40-70 percent of patients [Hunter *et al.,* 2004]. Predominant symptom is diarrhoea, watery, sometimes mucous but rarely bloody, sometimes profuse (1-2 l/day in a small minority of cases, usually very young or old). Pathogenesis is associated with tissue/cell damage of the intestinal epithelium. The infection of *Cryptosporidium* into enterocytes may lead to an increase of membrane permeability, changes in the ion flux and apoptosis. These effects are associated with the secretory diaarhoea. The precise pathogenic mechanisms are not well understood [Chappell *et al.,* 2003; Percival *et al.,* 2004]. Other symptoms include nausea, abdominal cramps, vomiting, fatigue, loss of appetite, fever. Shedding of oocysts may continue after the cessation of the disease symptoms.
Immunocompetent individuals clear the infection and *Cryptosporidium*-associated mortality is rare among the immunocompetent population. Outbreak data indicate a mortality rate lower than 1:100,000 [Hoxie *et al*. 1997]. In immunocompromised patients with severely depressed immune responses, especially AIDS patients with CD4 counts below 50 cells/mm3, infection results in illness in almost every case, and diarrhoea usually is chronic and may be fulminant, leading to rapid death. Clifford et al [1990] found that cryptosporidiosis affected 10-15% of the AIDS patients, causing death in 50% of the cases. The introduction of antiretroviral therapy has largely reduced the impact of cryptosporidiosis on HIV-infected individuals.
In normal otherwise healthy subjects, infection is thought to be limited to the ileum. In severely immunocompromised patients, infection may spread throughout the intestinal tract,

to the colon as well as the proximal parts of the gut (the gastric mucosa, and oesophagus). Respiratory infection and even sinusitis have been reported in such patients as well.
Even though the infection appears to be restricted to the ileum in immunocompetent persons, the occurrence of non-intestinal sequelae (joint and eye pain, recurrent headache, dizzy spells and fatigue has been reported for infection with *C. hominis*, but not for infection with *C. parvum* [Hunter *et al.,* 2004].
The human feeding studies with *Cryptosporidium* showed a clear distinction between infection and illness. Infection and colonization of the pathogens within the intestinal tract, can occur without any symptoms of gastro-enteritis. This is consistent with the view of infection as a necessary, but not sufficient cause for illness. The occurrence of infection among exposed subjects could be controlled by factors different from those influencing the occurrence of illness among infected subjects. Available data indicate that in immunocompromised patients the probability of illness among those infected with *Cryptosporidium* may be near 1, whereas in immunocompetent subjects this probability may be much lower (around 0.4 in feeding studies, for instance). This does not mean that susceptibility to infection is also much higher in immunocompromised subjects.
The seroprevalence of antibodies to *Cryptosporidium* is high, from 25-35% in developed countries to 60% in regions with poor hygiene. This does not necessarily imply that infection rates for *Cryptosporidium* must be high. If the decay rate of antibody levels is slow, any seropositive subject may have encountered their infection a long time earlier in life.

5.3 DOSE RESPONSE ANALYSIS

5.3.1 Human feeding studies

A series of human feeding studies was done at the University of Texas [Chappel *et al.,* 2003]. Before enrolment, volunteers were screened for the presence of antibodies to *Cryptosporidium*. Each volunteer then underwent a complete physical examination to find any illnesses or immunodeficiencies. Subjects who were in contact with subpopulations at risk (very young, very old, immunodeficient) were excluded. After eight hours of fasting, each volunteer received a single dose of oocysts in a gelatine capsule. Subjects were monitored intensively for 14 days after challenge (daily physical examinations, collection of all stools) and less intensively for the following 4 weeks (three visits a week).
Inocula were prepared from three different sources, all propagated in neonatal calves (meaning that they were genotype 2 or *C. parvum* oocysts). The Iowa and UCP isolates were obtained from naturally infected calves; the source of the TAMU isolate was an infected foal [details in Okhuysen *et al*. 1999]. The Moredun isolate was from cervine origin [Okhuysen *et al.* 2002]. After amplification in calves, fecal preparations were purified and stored in potassium dichromate buffer on ice. Before challenge, the preparations were safety tested for the presence of any other pathogens and resuspended in PBS. The oocyst concentration in this suspension was carefully adjusted, and checked by direct counting (haemocytometer, at least 6 replicate counts). Viability of oocysts in the inocula was ascertained by excystation

(80% or higher) and infectivity in neonatal mice. Oocysts were used within 6 weeks of their production in the calfs.
Infection has been defined as either detection of oocysts in the faeces 36 hours or later after challenge, or the presence of clinical cryptosporidiosis. Detection of faecal antigen (DFA) is not sensitive, and there may be false negative results. Subjects who did not excrete oocysts in detectable amounts but clearly displayed symptoms characteristic for cryptosporidiosis were therefore scored as infected. This has been called the clinical definition of infection.
The Iowa, UCP, TAMU, and Moredun isolates were tested in human volunteers who did not have pre-existing anti-*Cryptosporidium* IgG antibodies. Widely different ID_{50}s (estimated dose to infect 50% of the exposed population) were found, indicating considerable differences in infectivity among these isolates. Pathogenicities (attack rates of acute gastro-enteritis) also appeared to vary among isolates. All four isolates were capable of causing illness in healthy, immunocompetent human subjects. Incubation periods and durations of illness periods were similar for these isolates.
Nineteen of the 29 volunteers who had been challenged with the Iowa isolate were available to undergo rechallenge, with a single dose of 500 oocysts. Of these 19, infection was observed in 15 in the first challenge and no infection was observed in 4. The rechallenge did result in the same probability of occurrence of diarrhoea symptoms as the first challenge, indicating limited protective immunity against infection and illness. The number of oocysts shed by infected volunteers was lower than in the first challenge, resulting in alower risk of secondary transmission. However, when the same Iowa isolate was tested in a different group of volunteers with or without a pre-existing serum IgG to *C. parvum*, the ID_{50} was 14-20 fold higher in the volunteers with pre-existing IgG and illness was significantly associated with the highest doses.
To obtain a dose-response relation, the obtained data on infection were analyzed as a binary (yes/no) response with the single hit model for microbial infection [Teunis & Havelaar, 2000].

5.3.2 Hit theory for infection

The conceptual basis for the infection model is the observation that exposure and infection are conditional events. At physiologically meaningful concentrations, exposure to pathogenic micro-organisms usually implies that only a small, discrete number of particles is present, as opposed to toxic substances. A lethal dose of botulinum toxin A, one of the strongest toxins known to man still contains several hundreds of millions of molecules of the toxin. In contrast the ID_{50} of many infectious pathogens is a few hundred particles, and sometimes much lower. Therefore exposure to a low dose may be associated with a considerable probability that not a single organism has been ingested, in which case infection is not possible. For infection to occur, one or more oocysts must have been ingested, one or more of these ingested pathogens must have survived to reach a site suitable for growth, and, having reached this site, the sporozoites must still have been in a condition to infect host cells and complete the life cycle. When the ingested dose consists of only few pathogens, and/or the probability that they survive their journey through the digestive tract is low, the

success of infection may depend on a small number of organisms. The particulate nature of the inoculum is a property that is unique to microbial pathogens, and has a profound influence on the dose response relation, especially at low doses.

As most detection assays in microbiology are destructive, numbers of micro-organisms in a sample can usually only be estimated as an expected number. The dose, the ingested number of organisms, is treated as a Poisson sample with the estimated (or target) dose as parameter. Different doses are obtained by dilution, thereby adjusting the dose estimate by the applied dilution factor. Given a certain expected dose D the probability of exposure, i.e. the probability of ingesting 1 or more organisms is

$$\Pr_{\exp}(D) = 1 - \mathrm{e}^{-D}$$

Any ingested organism encounters many different barriers to infection: host defences, several in each compartment of the human intestinal tract, some part of the intestinal immune system, others mechanical (peristalsis, diarrhoea) or chemical (low pH, bile, pancreatic juices,…) in nature. Only organisms that succeed in passing all the barriers without damage can attach to a suitable site on the intestinal epithelium and initiate infection. Noting that (usually) a single surviving pathogen can grow and start a colonizing population leads to the single hit model: survival of 1 or more organisms after passing all host barriers is a necessary and sufficient condition for infection. For *Cryptosporidium* oocysts the gut is a big place, and we may assume that survival is an independent process: survival of one oocyst does not depend on the numbers present (i.e. the dose). If we call p_m the probability of survival for any individual ingested oocyst then the probability of infection is

$$\Pr_{\inf}(D \mid p_m) = 1 - \mathrm{e}^{-p_m D}$$

the dose response parameter p_m acts as a scale factor for the dose. This is the well known exponential dose response relation, the basic equation of all hit theory models. Unfortunately this model is too simple. In particular, the assumption that the host-pathogen interaction can be characterized by a single fixed factor p_m is unrealistic: both pathogen and host properties are variable. The infectivity of the individual oocysts in an inoculum is not constant, due to slight differences in conditions prior to ingestion, genetic variation, ageing. Nor is the susceptibility to infection the same in any host, even from a seemingly homogeneous population like that used in clinical studies. Therefore, most commonly the parameter p_m is given a Beta distribution, to represent heterogeneity in the host-pathogen interaction. The resulting dose response relation

$$\Pr_{\inf}(D \mid \alpha, \beta) = 1 - {}_1F_1(\alpha, \alpha+\beta, -D) \approx 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha}$$

is known as the Beta-Poisson model for microbial infection. The latter, simplified form is most frequently used, it should be noted that this equation is only correct for certain parameter values ($\alpha << \beta, \beta >> 1$). In many practical cases this approximate relation may be used, however it should be noted that while the best fitting curve may comply with these parameter constraints, use of the approximate relation still produces incorrect uncertainty estimates leading to gross overestimation of the extrapolated risk at low doses [Teunis &

Havelaar, 2000]. In some cases both α and β appear to be very small: that is a special case, where the Beta distribution, describing heterogeneity in host-pathogen interaction, is bimodal, with part of the cases having a very high risk of infection, and others a very low risk. In other words: part of the host-pathogen encounters is associated with a very high risk, and the remainder has a very low risk, virtually zero. This is an interesting phenomenon, which we may interpret as partial immunity: a fraction of the population appears protected against infection (and unprotected subjects may be at high risk). The corresponding dose response relation looks different: a steep rise at low doses, and saturation at an infection probability below 1. Interestingly, this is observed in the dose response relations of some isolates (Figure 11).

Box: Heterogeneity in microbial dose response models

In the absence of any heterogeneity the shape of the dose response relation depends only on exposure (the 'Poisson' part). For a Poisson inoculum this produces an exponential dose response relation. Any heterogeneity added, for instance by assuming variation in infectivity parameter p_m or overdispersed inoculum (e.g. aggregation) produces a less steep dose response relation. The exponential relation is the steepest model in the hit theory family of functions. For example, if we take notice of the fact that any oocyst of *Cryptosporidium* contains 4 sporozoites, and these do not necessarily all have identical infectivities, this merely adds heterogeneity to the infectivity of a single particle (the oocyst): 1 oocyst might contain 0,1,2,3, or 4 viable sporozoites. Such heterogeneity is adequately modelled by using the Beta-Poisson relation.

One comment needs to be made: if action of the infectious particles is not independent (as assumed in the single hit model), for instance if there is cooperation (a dose twice as high leads to a more than twofold increase in infectivity) the dose response relation is steeper. In the absence of heterogeneity an elegant demonstration of cooperative effects (like quorum sensing) might be found in testing whether the observed dose response relation is steeper than the exponential model. Unfortunately, in the real world heterogeneity is always present and we cannot discriminate cooperative interaction from heterogeneity: one tends to make the relation steeper, the other less steep. Any effect of cooperation might be countered by a certain amount of heterogeneity producing a relation with arbitrary slope. In statistics this is called 'unidentifiability'.

5.3.3 Pathogen factors: variation in infectivity among isolates

Data from each of the four isolates (Iowa, TAMU, UCP and Moredun) can be used for fitting dose response models. This produces four relations, with considerable differences in shape and location, see Figure 11, which begs the question how to use this information to make predictions of the infection risk for an unknown environmental isolate, as required in microbial risk assessment. Any of the individual dose response relations provides

information about the variation in infectivity of that particular isolate, but we would like to characterize the variation *among* these isolates in such a way that we can extrapolate to any type 2 oocyst sample.

The proper approach here is to analyze these different dose response data with a two-level model, the lower hierarchical level representing variation in single isolates, and the upper level representing variation among isolates. The Beta-Poisson dose response model is used, as in the simple one-level case, but now the parameters (α, β) are assumed to be taken from (joint) distributions, describing the 'between isolates' variation. This allows us to generalize the heterogeneous infectivity to the 'group' level for isolates of type 2, treating the four data sets as an n=4 sample from the presumed population of environmental type 2 oocysts. Instead of a single parameter set we now have to deal with a distribution, which may be interpreted as the frequency distribution for dose response relations from type 2 oocysts. Sampling from this type 2 'group' distribution produces a predictive dose response relation (Figure 11B), which is suitable for use in quantitative risk assessment.

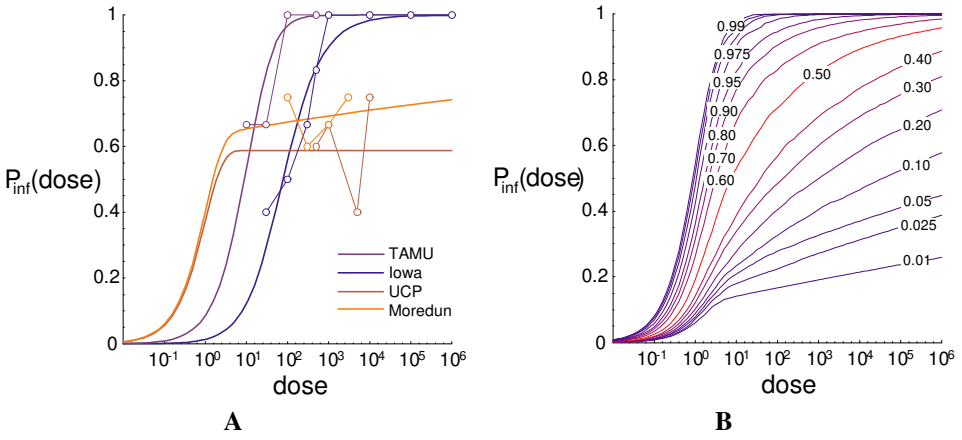


Figure 11. **A.** *Dose response relations for the four isolates TAMU, Iowa, UCP and Moredun. Note that the shape of the UCP and Moredun dose response relations could be interpreted as showing evidence for partial immunity.* **B**. *Quantile contours of the predicted dose response relation generalized from the 4 curves in A.*

The graph in Figure 11B suggests that the predicted distribution of dose response relations appears to cover a wide range of infectivities. This may be put into perspective by looking at

the range of predicted infectivities extrapolated to the low doses that may often be prevalent in drinking water sources. The graph in Figure 12A emphasizes once again that at low doses the exposure part of dose response relations becomes dominant: overall uncertainty in the predicted infectivity may be about 10-fold, not higher. We can also interpret the dose response parameters as describing the distribution of p_m, the infectivity of a single particle (oocyst). Figure 12B shows a graph of the predicted distribution of the expected value of p_m, or the mean of its (Beta) distribution.

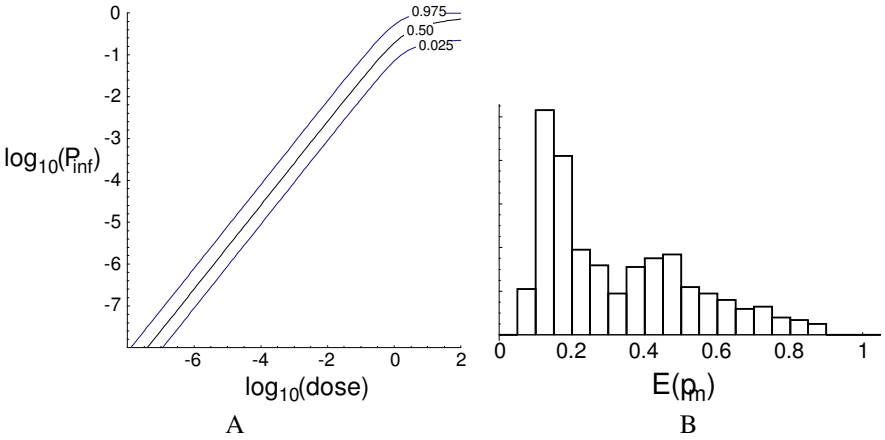


Figure 12. **A.** *Low dose extrapolated dose response relations for the four isolates TAMU, Iowa, UCP and Moredun.* **B**. *Histogram of the predictive distribution of the expected value of the single particle infectivity p_m. Note that this distribution covers almost the entire range (0..1).*

5.3.4 Host factors: immunity and susceptibility to infection

The high seroprevalence of anti-*Cryptosporidium* antibodies indicates that exposure to *Cryptosporidium* oocysts happens to most people at least once. In the challenge studies not all exposed subjects became infected or ill which raises the question whether there could exist some protective response resulting from infection (or illness). Interestingly, a rechallenge study with the Iowa isolate seems to indicate that if there is such a protective response, its duration is shorter than 12 months. On the other hand, when the same isolate was given to a study population of human subjects selected for high anti-*Cryptosporidium* IgG levels, with the same Iowa isolate, there was clear evidence of decreased infectivity compared to the prior study using seronegative volunteers [Chappell *et al*. 1999]. High IgG

levels could indicate a recent episode of cryptosporidiosis (or infection by *Cryptosporidium*), and may thus be associated with a protective response.

If we could describe the relation between IgG levels and susceptibility to infection in a quantitative relation this could be useful for quantitative risk assessment. Serum IgG concentrations can be measured in the general population, and if we could use individual IgG levels as a proxy for susceptibility we may at least partly control host variation in the general population.

In hit theory model for microbial infection the interaction between host and pathogen is contained in the parameter p_m. If we want to incorporate a covariable like IgG level into the model, this parameter can be made dependent on that covariable. Since p_m is a probability a logistic model lies at hand, so that ultimately we have a logistic relation nested into the exponential dose response model [Teunis *et al*. 2000].

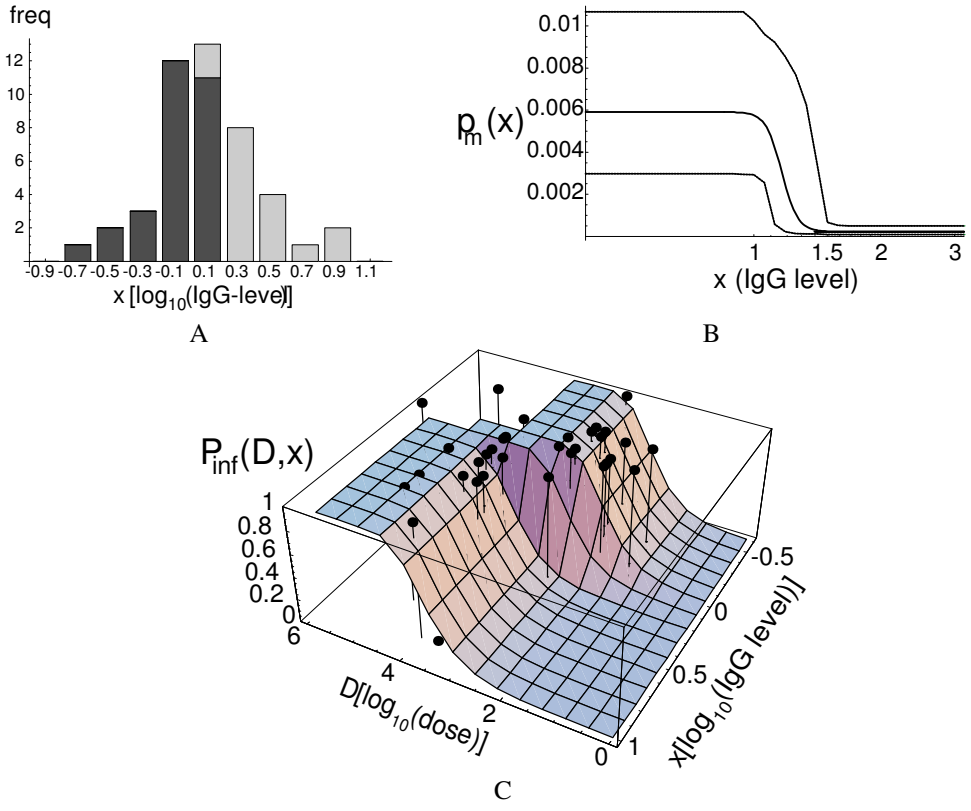


Figure 13. IgG-dependent dose response for infection by Cryptosporidium parvum (Iowa isolate). A Histogram of IgG levels in subjects prior to challenge (dark: low IgG-study, light: high IgG-study). B Dose response parameter p_m, as a function of (log-) pre-existing anti-crypto-IgG for the combined High + Low IgG data. Hatched: MCMC-based 95% limits. C. Best fitting dose relation for the probability if infection as a function of dose and IgG-level.

When data from the two studies with the Iowa isolate are combined we have a data on infection in subjects with a wide range of (baseline) IgG levels, as shown in Figure 13A. Fitting the IgG-dependent dose response model results in a function for the infectivity parameter p_m which shows clearly that there is a protective effect associated with high levels of baseline (pre-challenge) IgG. When such a model is applied to the data obtained in subjects with high pre-existing IgG levels, there is a clear indication of decreasing infectivity with increasing IgG levels (Figure 13B). Figure 13C shows the dose response relation, which is now a function of two variables, dose and baseline IgG level.

5.3.5 From infection to illness

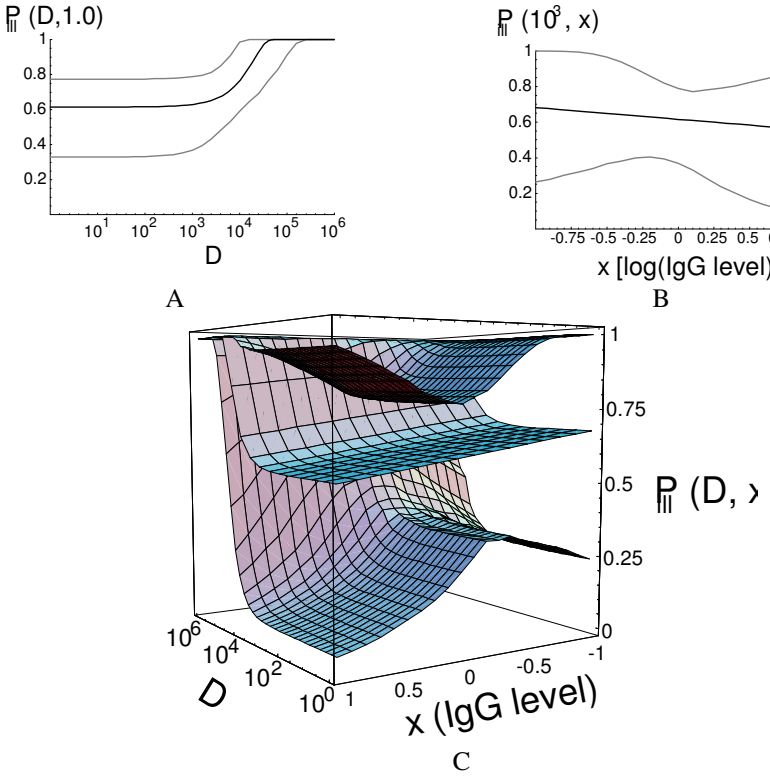


Figure 14. Dose and IgG dependent conditional illness model (illness among infected subjects) for illness among subjects challenged with various doses of the Iowa isolate of Cryptosporidium parvum. A. Conditional probability of illness at IgG =1.0 (log(IgG)=0) as a function of dose. B. Conditional probability of illness at a dose of 10^3 oocysts as a function of log(IgG). C. Conditional probability of illness as a function of both dose and log(IgG), with 95% predictive intervals.

Just like infection is conditional on exposure, illness is conditional on infection: when there is not a colonizing, metabolizing population of pathogens a host cannot become ill. During infection, parts of the intestinal tract contain many living pathogens. By considering the duration of infection as reflecting the balance between the colonization potential of the pathogens and the potential of the host for clearing the infection, a dose response model for illness in an infected host can be formulated [Teunis *et al*. 1999].

Since we have data on illness as a function of both dose and baseline IgG (again for the Iowa isolate), this model can also be made dependent on baseline IgG levels, like the infection model. Figure 14 shows some results, note that both dose and IgG-dependence are rather weak [Teunis *et al*. 1999]. Therefore alternatively, any infected subject may be assumed to have a fixed probability of becoming ill. This probability appears to be high, at least in these clinical studies, with healthy immunocompetent volunteers. For the Iowa isolate, the average probability of illness given infection was 0.65 (13/20) in low IgG subjects and 0.83 (10/12) in high IgG subjects. With the two other isolates, TAMU and UCP, no asymptomatic infections could be detected, so the probability of illness given infection is 1.0 for these isolates.

6

Risk characterisation

6.1 GENERAL APPROACH

Risk characterisation is the combination of the information about the probability of exposure to *Cryptosporidium* and the health effect of this exposure. Several authors have described the approach to this combination of exposure assessment and effect assessment in water supply [Regli *et al.,* 1991; Medema *et al.,* 1995; Haas *et al.,* 1996; Gale, 1996; Teunis *et al.,* 1997; Teunis & Havelaar, 1999; Haas *et al.,* 1999; Gale & Stanfield, 2000; Gale, 2002]. The information that is collected on the concentration of *Cryptosporidium* in drinking water, either by the direct method (Par. 4.2) or the indirect method (Par. 4.3), is combined with the consumption data to determine the probability that a consumer ingests one or more *Cryptosporidium* oocysts in a certain period. To determine the health risk associated to this exposure level, the exposure data are combined with the dose-response data (Chapter 5). This yields a probability of infection. This can be translated into a risk of illness and even risk of death if morbidity and mortality data are available (Figure 15). The probability of illness, the probability of death and the severity of the illness can be combined into "burden of disease"-measure, such as the Disability Adjusted Life-Years [Havelaar & Melse, 2003], so the health risk of *Cryptosporidium* through drinking water can be compared to other transmission routes, other pathogens or even other types of health risk in the society.

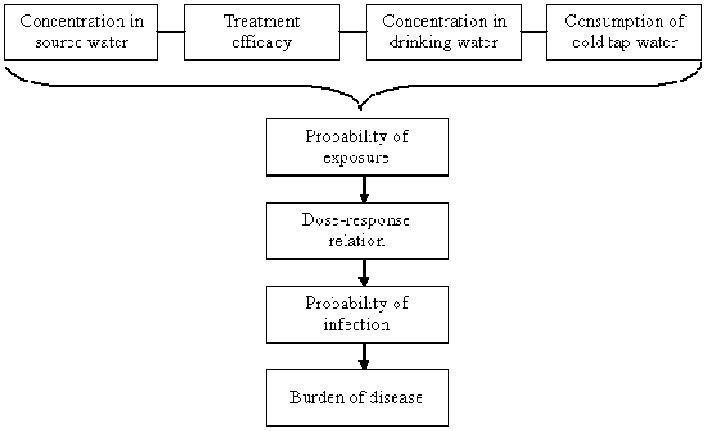


Figure 15. Integration of exposure and effect assessment to characterise the risk of Cryptosporidium through drinking water (from WHO, 2004).

6.2 RISK ASSESSMENT OF *CRYPTOSPORIDIUM* IN DRINKING WATER

The first quantitative microbial risk assessment studies on drinking water were conducted on viruses and *Giardia* [Regli *et al.,* 1991]. When the dose-response data from the first human volunteer study on *Cryptosporidium* [DuPont *et al.,* 1995] became available, several authors have performed QMRA for *Cryptosporidium* in water supply (Table 9).

The overview of QMRA-studies for *Cryptosporidium* in water supply illustrates several issues:

1. QMRA studies were conducted to:

- balance the health risk of *Cryptosporidium* in ozonated drinking water to the health risk of bromate formation by ozone [Havelaar *et al.,* 2000]. For the assessment of exposure to *Cryptosporidium*, they used raw water monitoring data on *Cryptosporidium*, data on the removal of anaerobic spores by conventional treatment and an ozone disinfection model (the Hom model published by Finch *et al.,* 1993) and a bromate formation model. The ingested dose of oocysts and bromate ions was translated to DALY's to allow comparison of the microbiological and chemical health risk. In their scenario, the health benefits of micro-organism inactivation by ozonation outweighed the health losses by bromate formation.
- demonstrate the need for additional treatment with UV [Aboytes *et al.,* 2004]. They used monitoring data of *Cryptosporidium* in treated water, using a cell-culture-PCR technique to determine the concentration of infectious oocysts in treated water.

Table 9 QMRA studies on the risk of *Cryptosporidium* in public water supply.

Authors	Exposure assessment	Effect assessment	Outcome	Type	Probability of infection average/95%-range
Medema *et al.,* 1995	*Cryptosporidium* in source water, recovery data [LeChevallier *et al.,* 1991],, viability data [LeChevallier *et al.,* 1991], removal of oocysts by full scale conventional treatment systems, [LeChevallier *et al.,* 1991], tap water consumption data [Roseberry & Burmaster, 1992]	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Probabilistic	3.6×10^{-5} [a] ($3.5 \times 10^{-7} – 1.8 \times 10^{-3}$)
Rose *et al.,* 1995	*Cryptosporidium* in treated water [LeChevallier *et al.,* 1991]	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Point estimates	5.0×10^{-2} ($4.4 \times 10^{-3} – 1$)
Rose *et al.,* 1995	*Cryptosporidium* in ice prepared from tap water at the time of an outbreak, the latter corrected for the effect of freezing/thawing (90% loss of detectable oocysts) and for the recovery	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Point estimates and comparison of observed and expected illness cases	-
Havelaar *et al.,* 1996	*Cryptosporidium* in source water, recovery data, removal of anaerobic spores by conventional treatment, NL cold tap water consumption data	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Probabilistic	1.3×10^{-4} [a] ($10^{-5} – 10^{-3}$)
Teunis *et al.,* 1997	*Cryptosporidium* in source water, recovery data, viability data [LeChevallier *et al.,* 1991], removal of anaerobic spores by conventional treatment, NL cold tap water consumption data	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Probabilistic	1.3×10^{-4} [a] ($4 \times 10^{-5} – 4 \times 10^{-4}$)
Teunis & Havelaar, 1997	*Cryptosporidium* concentration in source water [Atherholt *et al.,* 1998], recovery data [LeChevallier *et al.,* 1998], viable type morphology [LeChevallier *et al.,* 1991], removal by storage [Teunis *et al.,* 1997], removal of anaerobic spores by conventional treatment, NL cold tap water consumption data	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection, illness & DALYs	Probabilistic	No treatment failure: 2.0×10^{-12} 95%: 2.8×10^{-10} Treatment failure: 1.5×10^{-8} 95%: 2.1×10^{-6}
Perz *et al.,* 1997	Assumed concentration of *Cryptosporidium* in tap water, consumption of tap water [Roseberry & Burmaster, 1992], reduced by 40% for cold tap water consumption and by a further reduction of 33% for AIDS patients	Volunteer study with the Iowa strain [DuPont *et al.,* 1995], assumed three-fold higher infectivity for AIDS patients	Probability of infection and illness (probability of illness 0.5 for general population and 1.0 for AIDS patients). Estimated reported cases in general and AIDS population	Point estimates, using two assumed concentrations of *Cryptosporidium* in tap water	$1.0 \times 10^{-3/-2}$ in general population $2.1 \times 10^{-3/-2}$ in AIDS population
Havelaar *et al.,* 2000	*Cryptosporidium* in source water, recovery data, viability data [LeChevallier *et al.,* 1991], removal of anaerobic spores by conventional treatment, Hom model ozone inactivation [Finch *et al.,* 1993], NL cold tap water consumption data.	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	DALY	Probabilistic, comparing *Cryptosporidium* to	1.0×10^{-3} [a] ($7.6 \times 10^{-4} – 1.5 \times 10^{-3}$)

	The exposure was compared to the exposure to bromate that was formed in the ozonation.			bromate burden of disease	
Haas *et al,*1999 Haas 2000 J Food Protect 63:827	*Cryptosporidium* concentration in ice manufactured from tap water during an outbreak, estimation of the inactivation by freezing and thawing, estimation of the duration of the contamination (on onset of cases), attack rate during the outbreak, tap water consumption data [Roseberry & Burmaster, 1992]	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Point estimate, comparing expected and observed illness	1.1×10^{-2} [b]
Haas *et al,*1999	*Cryptosporidium* concentration in distributed water during an outbreak, estimation of the duration of the contamination (on onset of cases), attack rate during the outbreak, assumed 1 litre tap water consumption	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Point estimate, comparing expected and observed illness	3.6×10^{-4} [b]
Gale, 1998; Gale, 2000	*Cryptosporidium* in source water [Hutton *et al.,* 1995] and removal of oocysts by full scale conventional treatment systems, [LeChevallier *et al.,* 1995], data on heterogeneity	Volunteer study with the Iowa strain, including immunity	Probability of infection		1.5×10^{-3} [b]
Haas & Eisenberg, 2001	*Cryptosporidium* in different source watersheds, unfiltered system with chlorination, so removal/inactivation by treatment assumed as 0, tap water consumption data [Roseberry & Burmaster, 1992]	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Point estimate and probabilistic	1.2×10^{-2} 1.2×10^{-3} $(1.2 \times 10^{-4} – 7.7 \times 10^{-2})$
Medema *et al.,* 2003	*Cryptosporidium* in source water, recovery data, removal of anaerobic spores by conventional treatment, NL cold tap water consumption data	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Point estimate	$1.1 \times 10^{-3} – 3.5 \times 10^{-2}$
	Cryptosporidium in source water, recovery data, removal of bacteriophages by soil passage and of *Cryptosporidium* in soil column studies, NL cold tap water consumption data	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Point estimate	0
	Cryptosporidium in source water, recovery data, viability and genotype data, removal of anaerobic spores by conventional treatment, NL cold tap water consumption data	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Probabilistic	$<1.0 \times 10^{-4}$ with 91% certainty
Westrell *et al.,* 2003	*Cryptosporidium* in source water, removal of particles by conventional treatment, inactivation by disinfection [Korich *et al.,* 1990; Finch *et al.,* 1997], removal of oocysts by membrane filtration [Hirata & Hashimoto, 1998; Adham *et al.,* 1998]	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Probabilistic	Normal operation: 6.0×10^{-4} [a] $(6 \times 10^{-6} – 4 \times 10^{-2})$ Filtration error: 4.0×10^{-5} [a] $(6 \times 10^{-7} – 2 \times 10^{-3})$
	Cryptosporidium in sewage, reports of the water supply on treatment failure and contamination incidents in the distribution network	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Probabilistic	Reservoir contamination: 7×10^{-7} [a] $(2 \times 10^{-8} – 2 \times 10^{-6})$
Masago *et al.,* 2002	*Cryptosporidium* in source water [Hashimoto & Hirata, 1999], effect of rainfall, viability data [LeChevallier *et al.,* 1991], failure model for removal by conventional treatment, NL cold tap water consumption data	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Probabilistic	2.0×10^{-4} [a] $(2.5 \times 10^{-5}$ [c] $– 2.5 \times 10^{-3})$

Gale, 2002	Theoretical assumptions in scenario studies of treatment by-pass or failure	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Probabilistic	-
Pouillot *et al.,*2002	Assumed concentration in distributed water, recovery data, viability data (expert knowledge), French cold tap water consumption	Volunteer study with the Iowa strain for both infection and illness [DuPont *et al.,* 1995], immunodeficient mouse model [Yang *et al.,* 2000]	Probability of infection and of illness for immunocompetent and immunodeficient persons	Probabilistic	At 2 oocysts/100 litre: 1.8×10^{-2} 95%: 5.4×10^{-2}
Pouillot *et al.,*2002	*Cryptosporidium* in distributed water, recovery data, viability data (expert knowledge), French cold tap water consumption	Volunteer study with the Iowa strain for both infection and illness [DuPont *et al.,* 1995], immunodeficient mouse model [Yang *et al.,* 2000]	Probability of infection and of illness for immunocompetent and immunodeficient persons	Probabilistic	2.1×10^{-2} 95%: 6.7×10^{-2}
Demotier *et al.,*		Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Probabilistic	
Fewtrell *et al.,* 2001	*Cryptosporidium* in source water, recovery data, *Cryptosporidium* challenge study of conventional treatment	-	Quality score of exposure assessment factors	Uncertainty analysis	-
Haas *et al.,* 1996 JAWWA 88:131	Calculation of a *Cryptosporidium* concentration that corresponds with the 10^{-4} probability of infection (3.27×10^{-5} oocysts per litre (95% CI: 1.8 – 6.4 x 10	Volunteer study with the Iowa strain [DuPont *et al.,* 1995]	Probability of infection	Probabilistic	(1×10^{-4})
Aboytes *et al.,* 2003	*Cryptosporidium* in filtered drinking water, recovery data, infectivity data (cell-culture PCR)	Volunteer studies with the Iowa, UCP and TAMU with Bayesian data-analysis [Messner *et al.,* 2001]	Probability of infection	Point estimate with confidence interval	8.2×10^{-3} 95%: 1.2×10^{-2}
EPA, 2005	*Cryptosporidium* monitoring data (ICR and beyond), recovery data, infectivity fraction, treatment performance credits, USDA consumption data	Volunteer studies with Iowa, TAMU, UCP, using different models	Probability of infection, illness, death and cost	Probabilistic with sensitivity analysis	Scenario evaluation Pre-LT2 filtered: 8×10^{-5} ($<10^{-6}$ – 0.02); unfiltered 0.02 (0.002 – ~0.5)

[a]Median; [b] Average daily risk of infection during the outbreak; [c] Minimum annual risk

- or for treatment optimisation [Masago *et al.,* 2002; Medema *et al.,* 2003].
- illustrate the value of QMRA [Medema *et al.,* 1995; Teunis *et al.,* 1997; Teunis & Havelaar, 2002; Medema *et al.,* 2003; Pouillot *et al.,* 2004].
- to evaluate the risk of cryptosporidiosis in different water supply and sanitation scenarios [Westrell, 2004].
- to evaluate the impact of failures in treatment and distribution on the health risk [Westrell *et al.,* 2003]. Failure reports were collected from operational logs/interviews. These failures were translated into an estimate of *Cryptosporidium* (and other pathogen) occurrence (which was the most uncertain step in this QMRA). They indicated that in this system, the health risk associated with normal operation was higher than from the very infrequent and short lasting reported incidents.
- to prioritise research needs [Gale, 2002], who illustrates how QMRA can be used to determine the relative significance of major, well-controlled and minor, less well-controlled routes of exposure and the impact of moments of reduced treatment performance.
- To perform a cost-benefit analysis of *Cryptosporidium* regulation that requires additional drinking water treatment for systems with relatively high levels of *Cryptosporidium* in source water [EPA, 2005].

2. Exposure assessment is in many studies hampered by incomplete "site-specific" data. The gaps in the site-specific data are filled by using data from the scientific literature. This is particularly true for the studies in the 1990's. As the use of QMRA progressed, more authors have collected site-specific information about most if not all steps in the exposure assessment.

3. Most studies used the dose-response data of the Iowa strain of *C. parvum* as published by DuPont *et al.* [1995]. Over the years, the dose-response relationships of more *C. parvum* strains have been published. One recent study on the risk of *Cryptosporidium* to fire fighters using recycled water used the dose-response data of the TAMU strain of *C. parvum* as this was the most infective strain [Deere & Davison, 2004]. In the current review, we present an approach for the use of a *C. parvum* dose-response relation, which is a combination of the dose-response data that are published for four different isolates of *C. parvum* (Iowa, TAMU, UCP and Moredun; see chapter 5).

4. The most frequently used health outcome is the probability of infection; a few studies also determined the probability of illness of the general population and the immunodeficient population [Perz *et al.,* 1997; Pouillot *et al.,* 2002]. Two studies calculated the DALY resulting from the waterborne transmission of *Cryptosporidium* [Teunis & Havelaar, 1997; Havelaar *et al.,* 2000].

5. Using the data of the Milwaukee outbreak, the calculated probability of infection/illness with QMRA was compared to the observed probability of illness in the outbreak as observed in the epidemiological investigations [Haas *et al.,* 1999; Haas, 2000]. The authors conclude

that the results of QMRA and epidemiological investigation are consistent. The analysis of the exposure of the Milwaukee residents to *Cryptosporidium* via tap water was hampered by the lack of timely measurements of *Cryptosporidium* in the contaminated water. Unfortunately, this is the rule rather than the exception in waterborne outbreaks. The concentration had to be inferred from oocyst concentrations found in samples of ice that was prepared at the time of the water supply contamination and was corrected for the expected loss of detectable oocysts after freezing/thawing. The exposure assessment was therefore not very certain. In addition, the reported magnitude of the Milwaukee outbreak [MacKenzie *et al.,* 1994] has been criticised by Hunter & Syed [2001]. They claim that the background prevalence of gastro-intestinal illness in the US is much higher (1.2 – 1.4 episodes per person per year, or 0.10 – 0.12 per person per month) than the prevalence used by MacKenzie *et al.* [2004] (0.005 per person per month). If the higher background prevalence were used, this would drastically reduce the estimated size of the Milwaukee outbreak.

6. The set-up of the QMRA's sometimes used point estimates, but more generally a probabilistic approach is used to be able to estimate the level of uncertainty of the calculated probability of infection or illness.

7. Between the different studies, the calculated probability of infection can differ considerably (see table 10). Within studies, the uncertainty of the risk estimate towards the higher health risk (illustrated by as the difference between the average or median risk and the 95% confidence limit) is limited to around a factor of 10.

6.3 TIERED APPROACH

Risk assessment is well-suited for a tiered approach and this is also commonly used in risk assessment practice, both in human health risk assessment and in ecological risk assessment. The tiered approach allows an effective interaction between risk assessment and risk management, starting with a crude risk assessment, usually based on limited information to determine the urgency of the perceived problem, to prioritise the risk of different water supply sites or scenarios and to determine the need of a more detailed study for a particular situation. This allows the effective allocation of resources to the sites or situations that give rise to the highest risk. There is no strict definition of the tiers, only that the initial QMRA is usually generic and simple and the specificity and complexity increase in subsequent tiers. Examples of QMRA studies with different levels of specificity and complexity are given in three cases studies.

The most basic (but also most important) QMRA is a screening level study. Starting with whatever information is available, a crude first evaluation is made. Usually, the available information is not specific to the system that is studied, but has to be extrapolated from the available scientific literature. For a surface water treatment system, the information on *Cryptosporidium* levels in source water can be derived from watershed use (see par. 4.3) and

for the water treatment processes default log-credits for the removal or inactivation of *Cryptosporidium* are also given in par. 4.3. So in its simplest form, a QMRA can be performed with only a generic description of the water supply system. For instance, a water supply system with a watershed that can be characterised as moderately polluted (according to the criteria in par. 4.3) and with off stream storage reservoirs and a conventional (coagulation/filtration/chlorination) water treatment system, has an expected concentration of *Cryptosporidium* of 0.1 per litre in source water and 0.5 + 2.5 = 3.0 logs removal (see Table 5 and Table 6). Hence, the estimated concentration of *Cryptosporidium* in drinking water is 1.0×10^{-4} per litre. With a consumption of cold tap water of 0.78 litre per day (3.49 glasses of 0.25 litre, par. 4.4) the average probability of exposure to *Cryptosporidium* is 8.7×10^{-5} per person per day. With the combined dose-response relation of the four *C. parvum* strains the probability of infection is estimated at 3.8×10^{-5} per person per day, which amounts to 1.4×10^{-2} (=1.4%) per person per year. An example of a practical application of such a screening-level risk assessment is given in case study 1, where a large water supply company uses the screening-level QMRA to prioritise risk management of their water supply systems.

The screening level assessment may show that the risks are negligible, without much scientific doubt. In that case, the screening-level risk assessment can be used to demonstrate the safety of the system. Setting up an more detailed study is not warranted. Or the screening-level risk assessment may highlight that the risk is unacceptably high, again without much scientific doubt. In Case Study 1, the screening-level risk assessment is used to justify the installation of additional control measures. Such a screening-level risk assessment is also very useful in comparing different scenarios for risk management, e.g. different water treatment options.

If the outcome of the screening-level risk assessment is that there may be a health risk that is not negligible, there is an incentive for a next iteration of the risk assessment, collecting site-specific data, for instance on the presence of *Cryptosporidium* in the source water or catchment. The QMRA is repeated with the new, site-specific information. The options for the outcome of this second-level QMRA are the same as for the first iteration. In general, a result of any risk assessment is the identification of which information is missing and the prioritisation of research needs [Gale, 2002].

The screening-level risk assessments usually work with point estimates of risk. The tendency is to use conservative or worst-case estimates, to “be on the safe side”. But worst-case estimates, by nature, may overestimate the risk and it is not clear to the risk manager what the uncertainty of the calculated risk is, only that the uncertainty will be towards the lower risk values (the nature of a worst case assumption). More helpful for the risk manager is to provide a range of risks (interval estimate) that denote the variability and uncertainty in the risk estimate. In the case of the screening-level risk assessment this can be achieved by using an average, worst and best case, to illustrate the range of the risk that can be deduced from the available information and the level of certainty that is embedded in the QMRA. An example of such a QMRA is given in Case Study 2.

Interval estimates require information about the variability and uncertainty. Variability is the result of intrinsic heterogeneity in the input of the risk assessment, such as the variation in *Cryptosporidium* concentration in source water over time, or the variation in the removal of particles by a filtration process over time. Variability can be characterised if sufficient data points are collected. Uncertainty is the result of unknown errors in inputs of the risk assessment, such as errors in the measurement of *Cryptosporidium* or the assumption that certain indicator organisms can be used to describe the removal of *Cryptosporidium* by filtration. Uncertainty can be characterised by specific research activities, e.g. to determine the recovery efficiency of the *Cryptosporidium* enumeration method or to compare the removal of *Cryptosporidium* to indicator organisms by filtration.
When sufficient data are available, a probabilistic risk assessment can be performed, where the input is described by statistical distribution functions to describe the confidence interval of the input itself and of the calculated risk. An example is given in Case Study 3.

6.4 CASE STUDY 1: SETTING PRIORITIES FOR RISK MANAGEMENT

6.4.1 Problem formulation

Suez Environnement operates through Lyonnaise-des-Eaux, a large number (>1700) of water systems in France. After the waterborne outbreaks of cryptosporidiosis reported in the USA and UK, Suez wanted to develop an approach to evaluate the risk of *Cryptosporidium* for each water system. Such an approach would allow Suez to:

- demonstrate compliance with the EU drinking water directive (which states that drinking water must be free of …parasites…, which in numbers…may constitute a potential danger to human health.);
- know if any of these systems was at risk to *Cryptosporidium*;
- prioritise investments (if needed).

Specific risk assessment goal
What is the risk of *Cryptosporidium* in the (>1700) water systems?

6.4.2 Hazard identification

The risk assessment was focussed on *Cryptosporidium*, because of the absence of specific therapy against this microorganism, making it hazardous for immunocompromised consumers, and because of its resistance to chemical disinfection. A questionnaire was sent to the operators of each system, inquiring about the volume of water produced, the type of source water used, including information on the type of environment (urban, rural, presence of cattle etc.) and data on general water quality parameters (coliforms, turbidity, ammonium

and nitrate), and about the type of treatment processes. The returned information covered treatment facilities that supply 9 million people with drinking water.

6.4.3 Exposure assessment

Several source water systems discriminated on the basis of the data on source waters, source water environment and water quality: groundwater systems, groundwater systems under the influence of surface water, surface water systems and systems with drinking water that was a blend of the former systems. Information was obtained from a previous study aiming at measuring the occurrence of *Cryptosporidium* in surface water and in groundwater (Figure 16). These literature data were used to estimate the concentration of *Cryptosporidium* in each of the source water types identified, using a conservative estimation (Figure 17).

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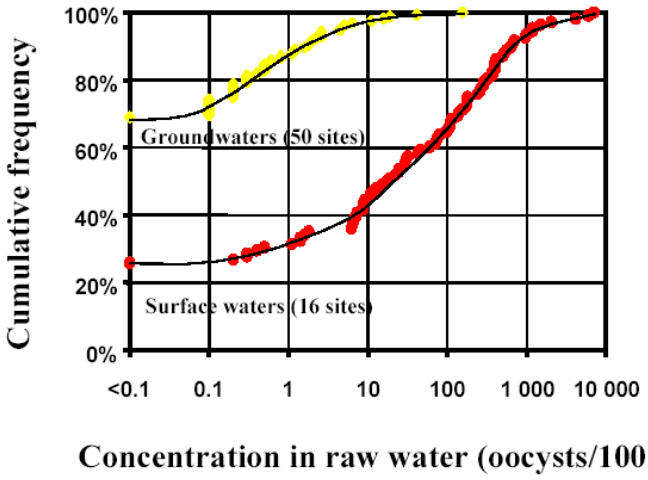


Figure 16. Cumulative distribution of the concentration of Cryptosporidium in surface waters and in groundwaters (CIRSEE – Suez Environnement data)..

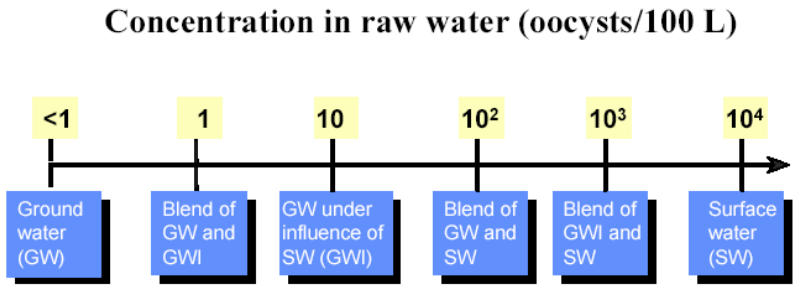


Figure 17. Estimated concentration of Cryptosporidium in source waters, based on the type of source water.

The removal or inactivation of *Cryptosporidium* by the treatment processes was also obtained from CIRSEE – Suez Environnement studies and collected from the scientific literature. Generic Log-credits were assigned to each of the treatment processes (Figure 18).

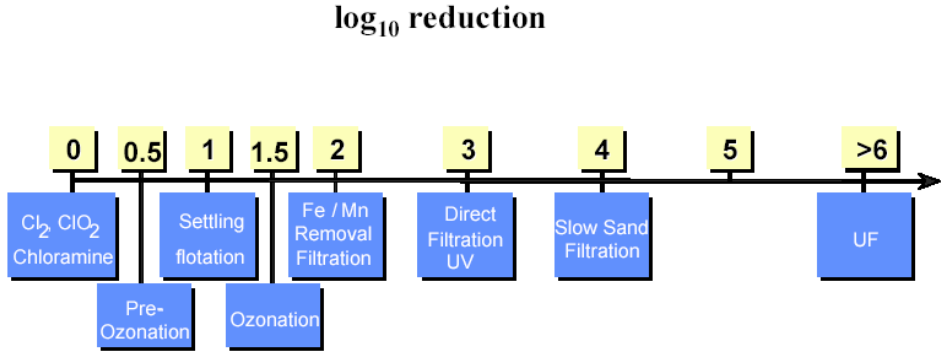


Figure 18. Log-credits for Cryptosporidium reduction by treatment processes.

The information about each treatment works was entered into a database with the facility to analyse the data.

6.4.4 Risk characterisation

For each system, a treatment performance target was defined according to the estimated concentration of *Cryptosporidium* in source water, in order to achieve one of these three levels of risk:

- A low level of risk was related to a *Cryptosporidium* concentration of 0.003 per 100 litres. This concentration was derived from the 10^{-4} probability of infection pppy, the suggested target for safe drinking water [Haas *et al.,* 1996] and using the exponential dose-response function of the Iowa strain.
- A medium level of risk was related to the analytical detection limit of *Cryptosporidium*, which was determined to be 0.3 oocyst per 100 litres. The associated probability of infection was 10^{-2} pppy.
- A high level of risk was arbitrarily set at 30 oocysts per 100 litre. The associated probability of infection was 0.6 pppy.

The treatment capacity of each treatment facility was compared to these treatment performance targets, and a level of risk was assigned to each facility accordingly (Figure 19).

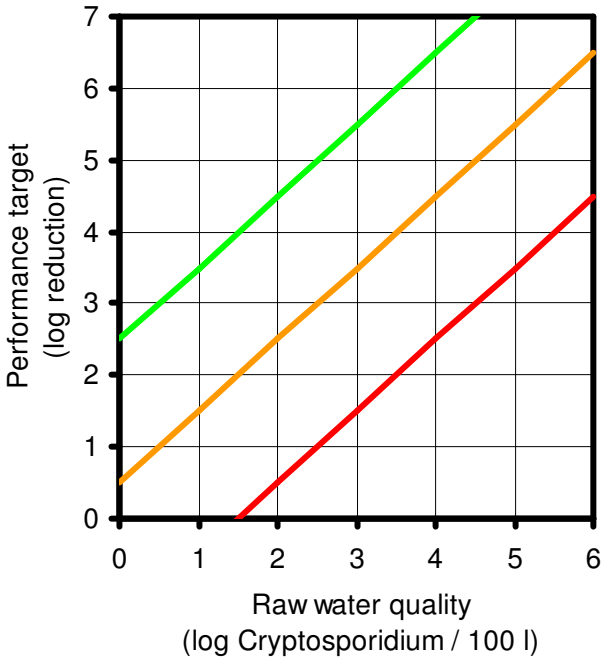


Figure 19. Matrix to determine the level of risk associated with a treatment facility, based on the estimated concentration of Cryptosporidium in source water and the performance (log-credits) of the treatment processes.

6.4.5 Risk management

The risk assessment highlighted that the sites at higher risk were primarily small systems (serving <5000 people) and groundwater systems that were under the influence of surface water.
To validate the outcome of the risk assessment, a *Cryptosporidium* monitoring program was conducted at selected sites from each of the risk categories. In this monitoring program, treated water samples were collected every two weeks for a period of at least 6 months and analysed for *Cryptosporidium* (and *Giardia*) and other water quality parameters: turbidity, coliforms, aerobic spores, temperature, pH, conductivity, UV transmission and ammonium. Additional samples were taken during turbidity peaks.
The results of the *Cryptosporidium* monitoring were consistent with the risk assessment; high risk sites showed the highest frequency of samples positive for *Cryptosporidium* and the highest concentrations were observed at these sites. No *Cryptosporidium* was found at any of the low risk sites and the medium risk sites gave intermediate results. There was also a good correlation between the presence of *Cryptosporidium* and high turbidity. The verification of the QMRA with the monitoring data was very valuable to convince the risk managers and owners of the water supplies that the QMRA results are a valid and good basis for setting risk management priorities.
The main risk factors for *Cryptosporidium* that were found were the presence of cattle in the catchment area, less than 99% compliance with the coliform standard and turbidity of >0.2 NTU in distributed water. The company has audited the high risk sites and, where necessary, has upgraded the treatment facilities in accordance with the local health authority. Furthermore, Suez has since then used the same risk assessment approach for the water works they operate in other countries.

6.5 CASE STUDY 2: EVALUATING A RISK SCENARIO

In this case study, no site specific information is available on *Cryptosporidium*, but data are inferred from literature.

6.5.1 Problem formulation

A proposal for a 33-lot unsewered, residential subdivision was lodged with a Shire Council in south-eastern Australia. The proposed subdivision was located on marginal grazing land in the catchment of a water supply reservoir. The reservoir supplied a population in nearby towns and surrounding areas of approximately 42,000. Under State planning laws, the local water utility was listed as a referral authority and needed to provide an approval for the development to proceed. When the utility refused permission for the development on the grounds that, being unsewered, it posed a threat to water quality in the reservoir; the property developer took the utility to court. The developer argued that best practice wastewater management and on-site treatment systems would ensure the protection of water supplies.

The utility countered this argument with a preliminary quantitative risk assessment and evidence that on-site treatment systems rarely met design performance standards due to poor maintenance. Under realistic operating conditions, the risk assessment showed that the threats to public health from *Cryptosporidium* exceeded the US EPA's Surface Water Treatment Rule (SWTR) baseline tolerable risk for domestic water supply of 10^{-4} cases of gastrointestinal illness per annum.

Specific risk assessment goal
Does the proposed development pose an intolerable risk to water supplies?

6.5.2 Hazard identification

In order to assess the additional public health risk posed by the proposed subdivision a spreadsheet model was developed based on *Cryptosporidium* oocysts (Table 10). *Cryptosporidium* was considered to be the pathogen most likely to pose a threat due to the resistance of oocysts to conventional water treatment. Oocysts resist disinfection in drinking water treatment plants and can survive for 3 months or more in freshwaters (Fayer *et al*. 1998). Consequently any additional sources of *Cryptosporidium* in water supply catchments are highly undesirable.

6.5.3 Exposure assessment

The key parameters in the model were the analytical laboratory recovery rate (i.e. the detection efficiency), the concentration of *Cryptosporidium* in the sewage treatment plant effluent, the percentage of effluent reaching the stream feeding the water supply reservoir, the average stream flow, the oocyst removal rate of the drinking water treatment plant, and the average volume of water consumed per person per day.
Each parameter is subject to some degree of uncertainty, however realistic values were chosen for the average case scenario. A sensitivity analysis was also conducted using best and worst case scenarios to give an indication of the upper and lower limits that could be expected. For reasons of simplicity, extreme values for some of the parameters in the sensitivity analysis were not chosen. The rationale for each of these decisions is discussed below.

Source water quality
Each of the properties on the subdivision were to rely on septic tanks or small aerated wastewater treatment plants (AWTS) for the disposal of household wastes, with either transpiration or evaporation as the means of final effluent disposal.
Average values for the removal of oocysts by conventional wastewater treatment plants can be estimated from the literature. For the purposes of this risk assessment it was assumed that the average treatment efficiency of the sewerage treatment systems for the proposed

subdivided plots was 2 $\log_{10}$ (i.e. 99%). Crockett and Haas (1997) present figures for the concentration of *Cryptosporidium* oocysts in raw sewage with the highest value from a limited survey showing a concentration of 290 oocysts per litre. Using this concentration and assuming a 2 $\log_{10}$ removal gives a value of 2.9 oocysts per litre. The effluent disposal systems recommended for the development were either conventional adsorption of the primary septic tank effluent in absorption trenches or trickling irrigation of secondary effluent, which has undergone treatment in an aerated wastewater treatment system (AWTS). Both of these systems could easily have a removal rate lower than the assumed value. State Wastewater Reuse Class A guideline level for parasites (i.e. the category which includes *Cryptosporidium*) is < 1 parasite per 50 L. While this is a desirable target on public health grounds, in practice it is very difficult to meet. Sewage Treatment Plants (STPs) and other means of treatment are expected to have significantly lower treatment efficiencies due to the poorer quality of the raw effluent they receive. With relatively fixed removal efficiencies, the concentration of oocysts in the effluent is likely to depend heavily on the load going to the STP. Consequently a concentration of 0.5 oocysts per litre for the best case scenario and 3 oocysts for the worst case were chosen.

The water usage for each lot was proposed to be 1200L/day. This value was chosen for the average case scenario. The percentage of effluent reaching the small creek that drained the proposed development was modelled at 10% for the average case. This was based on the fact that oocysts can survive in the damp conditions on irrigated pasture and could be washed by subsequent rainfall down the slopes of the site into the creeks. Soil analysis, rainfall levels and anecdotal evidence also indicated that there may be periods over winter were the soil becomes water logged. Automated irrigation control (e.g. rain sensors stopping irrigation when rain is detected) would help in reducing the risk of oocyst transport to the creek; however, there is little doubt that at certain times of year a significant quantity of oocysts would enter the creek.

Stream flow was modelled as the average flow in the creek (estimated from relevant Gauging Station Data), which was around 4 ML per day. A more sophisticated model (beyond the scope of this preliminary study) could include variation in stream flow, although it was not expected that this would alter the predicted magnitude of the probability of infection. Since the model was aimed at assessing the *increased* health risks due to the proposed development, the concentration of *Cryptosporidium* oocysts in the creeks upstream of the development was assumed to be zero[2]. Consequently, the estimated concentration of oocysts in the stream downstream is given by the total oocyst load divided by the total discharge (stream flow plus effluent).

[2] Another approach could have been to compare current land-use with proposed use, however, due to the marginal nature of the farmland, the continuity of current land-use was highly unlikely.

It is important to note that the turbidity of sample waters can greatly decrease WTP pathogen removal efficiency. Consequently highly turbid storm flows which may mobilise *Cryptosporidium* oocysts off wastewater irrigated pasture, stream beds, or water storage sediment surface and which increase turbidity, pose greater pathogen control problems.

Once the oocysts enter the stream they travel approximately 5 km before discharging into the reservoir. In the reservoir the storage time is likely to be in the order of some months, however, it is not known what proportion of oocysts that may enter the reservoir may die off and or settle out before being drawn off for treatment and drinking water supply. In shallow reservoirs even light winds can keep small particles in suspension and it is unlikely that there would be significant settlement of such small particles (4.2 to 5.4 μm for *Cryptosporidium parvum*, Arrowood 1997). Some particle flocculation may occur which may increase sedimentation rates, reducing the number of oocysts in the water column but the extent of this is unknown. In the absence of detailed information, a conservative approach was taken in assuming no die-off or settlement of oocysts entering the reservoir and that the concentration of oocysts reaching the WTP is the same as in the creek.

Recovery efficiency
The laboratory recovery rate for *Cryptosporidium* was set at 33%. This is based on the analytical laboratory's performance data and is an average value for recovery based on the particular analytical methodology that may be used and the quality of the water tested. Many factors, in particular high water turbidities can interfere with the analytical extraction processes, thus the recovery rate is somewhat variable.

Treatment efficacy
Small water treatment plants generally have a treatment process that includes coagulation, sedimentation and filtration and this is generally expected to remove up to 99% of oocysts (i.e. a 2 $\log_{10}$ removal rate) (Rose *et al.,* 1997). It is possible that the WTP could exhibit infrequent suboptimal performance (e.g. during storms, when oocyst numbers may be at their highest) so the WTP removal rate for oocysts has been modelled at 95% for the average case scenario.

Table 10. Additional risk of infection by Cryptosporidium posed by the subdivision and subsequent building of wastewater treatment systems consumers of treated drinking water sourced from reservoir. The average case, best case and worst case scenarios are presented.

		Sensitivity Analysis	
Item	Average	Best	Worst
Analytical laboratory recovery rate	33%	33%	33%
Likely concentration in effluent (no/L)	2.9	0.5000	3
Water use and discharge (L/Day) (all 33 Lots included)	39,600	39,600	39,600
Proportion of discharge reaching creeks	3,960	3,960	39,960
Stream flow (L/Day)	3,500,000	3,500,000	3,500,000

Assumed *Cryptosporidium* concentration in stream upstream (no/L)	0.0000	0.0000	0.0000
Assumed *Cryptosporidium* concentration in stream downstream (no/L)	0.003277	0.000565	0.033563
Treatment removal rate of *Cryptosporidium*	95%	99%	1%
Volume consumed per day (L)	1.95	1.95	1.95
Dose received (N)	0.00095865	0.00003306	0.19634422
Probability of infection (P_i) per day	4.47688×10^{-6}	1.54376×10^{-7}	9.16507×10^{-4}
P_i per annum	1.63406×10^{-3}	5.63471×10^{-5}	3.34525×10^{-1}

6.5.4 Risk characterisation

The above calculations give the estimated concentration of oocysts in domestic drinking water. The probability of infection, $P_{i,}$ per day is given by the equation:

$$P_i = 1 - \exp(-rN)$$

where r is the fraction of *Cryptosporidium* oocysts that are ingested which survive to initiate an infection and N is the daily exposure (i.e. actual number of oocysts ingested). For *Cryptosporidium*, r = 0.00467 and N = the number of oocysts in 1.948 L, the assumed daily ingestion rate (Rose *et al*. 1997). For the average case scenario $P_i = 4.48 \times 10^{-6}$. Multiplying this figure by the number of days in a year gives an annual risk of infection of 1.63×10^{-3}. This is the additional risk posed to consumers within the subdivision. It exceeds by a factor of 16 times the US EPA's Surface Water Treatment Rule (SWTR) baseline tolerable risk for domestic water supply and is clearly intolerable.

As stated, the models were based on the conservative assumption of no die-off of oocysts before reaching the WTP. If a die-off factor of 90% is incorporated into the calculations, the average and worst case scenarios for annual risk of infection would be 10% of the values shown in Table 10, however these would still exceed the SWTR tolerable risk criterion.

6.5.5 Risk management

On-site treatment system reliability

In addition to the above risks, there were a number of additional operational risks that have not been modelled or considered. These include the probability of spill incidents, breakdowns in irrigation systems, failure of secondary treatment if this option was chosen, increased water usage and subsequent flooding of the system, poor maintenance on septic tanks including structural break downs and leakage from the system, the effect of fire damage on plant performance and operation of irrigation system after vegetation loss following fire, and a range of other difficult to control events.

There is mounting evidence that maintenance of on-site treatment systems is frequently poor. In support of its case the water utility conducted monitoring of streams near existing unsewered towns in its area of jurisdiction. The results of these studies provided compelling evidence that on-site systems are commonly poorly maintained. Measurements made of a number of water quality parameters including nutrients, biological oxygen demand (BOD) and *E. coli,* showed that drainage from residential subdivisions, many of the size of the proposed development frequently recorded high levels of *E. coli*, BOD and nutrients. In some cases these levels were of such magnitude that they constituted a public health threat from direct contact.

On the basis of the arguments put forward by the water utility, the court - in this case an administrative tribunal chaired by two commissioners - found in favour of the utility and the development did not proceed. The value of the risk assessment study was to quantify the likely risk to water supplies. Despite the uncertainty surrounding appropriate values of many of the model parameters, simple worst case and best case scenarios were able to indicate the bounds on the uncertainty. More sophisticated risk modelling and uncertainty analysis would have narrowed the degree of uncertainty. However, the risks of the proposed development were of such magnitude that further risk modelling was unnecessary.

6.6 CASE STUDY 3: MEETING THE HEALTH-BASED TARGET

The use of point estimates for each component of the risk assessment has the advantage of simplicity and communication of the information to risk managers. However, the disadvantage of point estimates is that the information about variation and uncertainty is not taken into account, and point estimates give a false sense of certainty. The information about variation and uncertainty is important when risk management actions are decided upon, as they guide risk management to the most important areas of control and uncertainty.
The Markov Chain Monte Carlo method can be used to combine different probability distributions. Hence, the variability and uncertainty can be included in the risk assessment and the outcome is a probability distribution of the risk estimate which gives the confidence interval of the risk estimate. Using sensitivity analysis, it is possible to identify which information source contributes most to the overall uncertainty of the risk estimation.

The information on *Cryptosporidium* oocyst concentrations, recovery efficiency of the detection method, efficacy of treatment processes, distribution integrity, and the consumption of cold tap water may now be used to calculate daily doses for *Cryptosporidium*. To this end, samples are drawn from the fitted probability distributions for these factors using the Monte Carlo method. Resampling can provide information about multiple exposures [Teunis *et al.,* 1997]. Using the distribution of the daily dose and the dose-response the risk of infection and illness can be estimated. The outcome is a probability distribution of the probability of infection or illness per day that can be transformed to the annual risk.

6.6.1 Problem formulation

A Water Company in the Netherlands is the owner and operator of a plant for drinking water production from surface water. Given the occurrence of outbreaks of cryptosporidiosis through drinking water in neighbouring countries the water company and the drinking water inspectorate want to know if the population served by the treatment plant is adequately protected against cryptosporidiosis.
The new Dutch Drinking Water Act states that for pathogenic micro-organisms, a health risk should not exceed 1 infection per 10.000 consumers per year (VROM, 2001). There are precedents in the drinking water industry in the Netherlands where this risk level has been used to evaluate the safety of drinking water.

Specific risk assessment goal
Does this treatment plant produce drinking water that meets the 10^{-4} infection risk-level?

6.6.2 Hazard identification

The risk assessment is limited to *Cryptosporidium*. As the treatment contains chlorination it is argued that *Cryptosporidium* is likely to be the most significant health hazard. The outcome of the risk assessment is the risk of infection, since this is the health outcome that is incorporated in the regulation.

The water company uses water from a large, international river as source water. The river is contaminated by discharges of mainly treated domestic waste waters. During rainfall events, additional contamination is likely to come from run-off from agricultural lands and overflows of combined sewers. Treated sewage is not discharged in the vicinity of the abstraction site.
The river water is pre-treated by conventional treatment (coagulation, sedimentation and rapid sand filtration). The pre-treated river water is then transported to the coastal sand dune area and infiltrated in the sandy soil (by surface infiltration). The water is abstracted from the soil again by pumping wells at a distance of the infiltration ponds. The travel time of the water through the sandy aquifer is 60-100 days. After abstraction, the water is recollected in an open canal system and reservoir. This open system lies in a natural area, but becomes recontaminated by waterfowl, wildlife and grazing cattle. Measurements have shown faecal contamination to be present in the recollected water, esp. in winters when the bird load is high. *Cryptosporidium* has been detected in the recollected water and genotyping has indicated that at least part of the oocysts in this water is *C. parvum.* The recollected water is treated by rapid sand filtration, ozonation, softening, GAC filtration and slow sand filtration. The production of the plant is 180,000 m3/d.

Rapid sand filtration

The water is collected from the open reservoir and aerated by cascades. This aerated water is filtered over 56 separate filter units (total area of 2368 m2). With a filtration rate of 3.3-4.9 m/h the water is filtered over a bed with river sand (0.7-1.4 mm) of 1.3 m height (HRT = 16-24 min). The filters are back washed on based either on head loss or time of operation (96 h on an average).

Main disinfection
The water (8000-11,300 m^3/h) is disinfected with ozone. In winter and summer with a dose of 0.9 and 0.75 mg/l (counter current) resulting in CT-values of 3-4 and 1-2 (mg/l)*min, respectively. The CT is limited to control the formation of bromate. The water is treated in 5 separate units 778 m^3 each and the HRT is 15-24 min. Residual ozone concentration is <0.01 mg/l. The $T_{10}/T_{hydraulic}$ of the system is 60%. CT is regulated weekly by measurement of the ozone profile of the installation (5 sampling points), the UV-extinction and temperature.

Softening
After ozonation the water is treated with caustic soda (pH= 8.7-9.2) and sand grains in fluidized bed reactors (12; HRT= 1-2 min) to reduce hardness. After this process pH is set at a saturation index of -0.2 with hydrochloric acid.

GAC filtration
Softening is followed by granular activated carbon (Norit ROW 0.8) filtration in 20 separate filter units of two successive filter beds with a total HRT of 40 minutes (two times 20 min.). The surface area is 58 m^2 per unit of two filters, the bed height is 5 m (x2.5), the filtration rate 7-10 m/h and the HRT range 30-43 min. (8000-11,300 m^3/h). These filters are back washed once or twice per month with air (60 m/h) in summer and with water all seasons (20-40 m/h).

Slow sand filtration
Last stage of the post-treatment is slow sand filtration (25 filters). With a rate of 0.25 m/h (maximum of 0.5) the water is filtered through a filter bed (0.8-1.2 m; HRT = 190-290 min.) with mol and silver sand (0.15-0.6 mm). The filter bed is scraped with a frequency of once every two years, depending on the head loss, to maintain production capacity.

Post-disinfection
This plant does not use post-chlorination (drinking water is distributed without residual disinfectant).

Sludge treatment and back wash water reuse
The back wash water of the rapid sand filters and the GAC filters (3,800 m^3/d) is treated by in-line coagulation (1.4 g/ $FeCl_3$) and Dynasand filtration. There is of filter-to-waste period after back washing.

Operation diary for disturbances
There is a registration of disturbances, maintenance and incidents.

The treated water is stored in a 13,400 m^3 reservoir and distributed in a network. The yearly production is 65,000,000 m^3 per year for 600,000 clients.

6.6.3 Exposure assessment

Source water quality
The Water Company has conducted a monitoring programme of its source water over the period of several years. The source water was sampled each month for the presence of *Cryptosporidium*. 48 Samples of 100 – 200 litres were taken and concentrated and purified according to the EPA 1623 method. Fifteen of the 48 samples contained *Cryptosporidium* oocysts. The average concentration in the positive samples was 4.8 per 100 litres and the maximum was 19.3 per 100 litres. The concentrations in the winter season were highest.
The oocyst counts can be fitted with statistical distributions. The distribution is commonly skewed: a few samples contain a relatively high oocyst count while most samples contain no or very low oocyst numbers. Several authors have described fitting of statistical distributions to such *Cryptosporidium* concentration data-sets. Teunis *et al.,* [1997] compared the Poisson distribution, the Poisson distribution with added zero's and the Negative Binomial distribution to *Cryptosporidium* concentration data from an off-stream storage reservoir and concluded that use of the Negative Binomial distribution provided the best fit. The latter has been shown to give the best description of these river water data. Havelaar *et al.* [2000] used the Log-Normal distribution on these same data. Also Gale & Stanfield [2000] used the Log-Normal distribution to fit *Cryptosporidium* concentration data in raw water. The advantage of the Log-Normal distribution is that when the distribution of oocysts in source water is combined with the other elements of exposure assessment (treatment efficacy), the overall variance can easily be determined by simple summation of the variance of the individual elements. Teunis *et al.* [1999] used the Negative Binomial distribution to describe *Cryptosporidium* concentration data from LeChevallier *et al.* [1998], assuming that the individual *Cryptosporidium* count data were Poisson distributed, with the concentration varying between samples, following a Gamma distribution. The combination of these assumptions means that *Cryptosporidium* counts follow a Negative Binomial distribution. Medema *et al.,* [2003] and Pouillot *et al.* [2004] also used the Negative Binomial distribution to fit their *Cryptosporidium* concentration data.
A Gamma distribution was fitted to the *Cryptosporidium* counts in source water. Figure 20 shows a histogram of the data; the probability of no or low oocyst counts is high, and the probability of high oocyst concentrations is low. The figure also shows the probability density function (PDF) and the cumulative density function (CDF) of the fitted Gamma distribution, testing also for a Gamma distribution with a large α and small β and the reverse. The "average" Gamma distribution with the α of 1.18 and β of 20.94 was selected for inclusion in the QMRA.

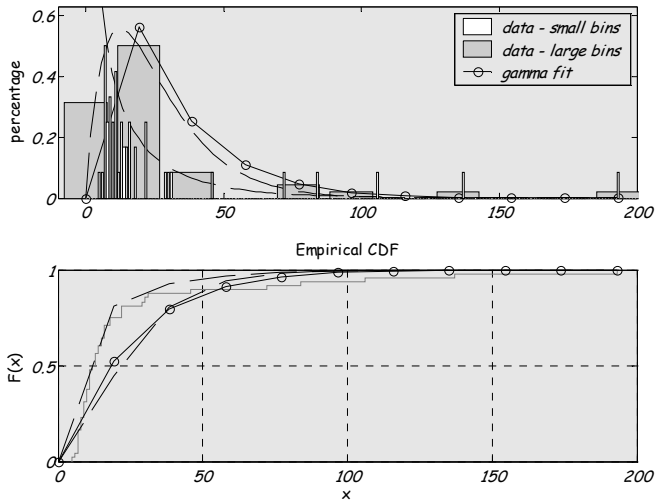


Figure 20. Frequency distribution (PDF and CDF) of Cryptosporidium concentration in source water, and the fitted Gamma distribution (X-axis shows Cryptosporidium concentration in oocysts per 1000 litres).

As stated before, the detection methods for *Cryptosporidium* in water have a low and variable recovery efficiency and the observed concentrations should be corrected for this recovery efficiency. Ideally, recovery data are available for every individual sample. If that is the case, the individual *Cryptosporidium* counts can be corrected for the recovery efficiency and the statistical distribution can be fitted to the corrected concentrations [Medema *et al.,* 2003]. If the recovery efficiency is not available on all individual samples, data on the recovery efficiency from a subset of samples can be used. If these are also not available, recovery data from quality assurance testing should at least be available, otherwise the laboratory has no way to demonstrate the efficacy of their detection method. The simplest approach is to correct all the concentration data with the average recovery efficiency [Gale & Stanfield, 2000, using data of Hutton *et al.,* 1995]; Havelaar *et al.* 2000]. Teunis *et al.* [1997] described a statistical method to describe the recovery efficiency. Assuming each oocyst in the water sample has a probability *p* of being recovered and this probability is not fixed but varies following a Beta distribution, the recovery data should be fitted to a Beta-Binomial distribution. The recovery efficiency and *Cryptosporidium* concentration data are combined with Markov Chain Monte Carlo methods. Using this approach, the variation in the recovery efficiency is taken into account and the uncertainty that is introduced by the variable recovery efficiency can be quantified [Pouillot *et al.,* 2004]. This is of importance when the outcome of the overall risk assessment is to be interpreted in terms of the risk management

actions that are required. Uncertainty introduced by the detection method requires another approach than variation introduced by fluctuations in treatment efficacy.
The recovery of the method that was used to enumerate the *Cryptosporidium* oocysts in source water was evaluated for 30 surface water samples at different sites (including this site) over the same years as the monitoring at this site. The average recovery efficiency was 22.0% (95% CL: 2.3 – 71%). Figure 21 shows a histogram of the data (expressed as fraction recovered) and the PDF and CDF of the fitted Beta and Exponential distribution. The Exponential distribution fitted best to the data and was selected for the QMRA.

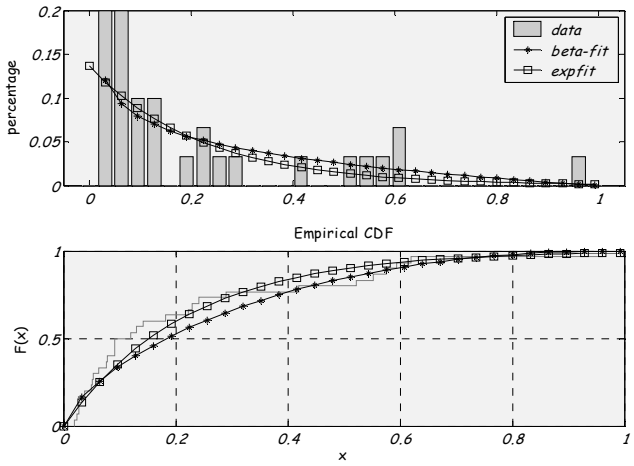


Figure 21 Frequency distribution (PDF and CDF) of the recovery efficiency of the Cryptosporidium concentration method, and the fitted Beta and Exponential distribution.

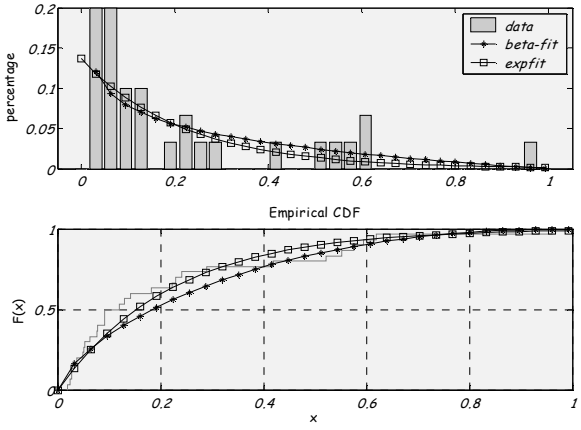


Figure 22. Frequency distribution (PDF and CDF) of the Cryptosporidium recovery efficiency, and the fitted Beta and Exponential distribution.

Treatment efficiency

To get a first idea of the level of protection of the drinking water, an approximate assessment of the treatment efficiency was conducted, using the default treatment efficiency as given in par. 4.3. The rapid sand filtration and GAC filtration would both remove 0.5 log, the slow sand filtration 2 log and the ozonation 0.25 log in winter to 1 log in summer. This would result in an estimated concentration of oocysts in drinking water (corrected for the recovery) of 0.7-3.9 x 10^{-4} per litre. With an average daily consumption of cold tap water of 0.78 litre per person the average probability of exposure would be 0.6-3.3 x 10^{-4} per person per day. This would result in a probability of infection of 0.26-1.4 x 10^{-4} per person per day (using the combined dose-response relation of the 4 *C. parvum* isolates (chapter 5), which corresponds to an annual risk of infection of 0.9-5 x 10^{-2} per person, above the maximum acceptable risk of infection that is present in the Dutch regulation.

This called for a more comprehensive and site specific assessment of the treatment efficiency. The water utility had data available on the presence of spores of sulphite-reducing clostridia at different stages of the treatment train. The removal of these spores was used as a model for the removal of *Cryptosporidium* oocysts by the treatment processes. For the slow sand filtration, specific data on *Cryptosporidium* removal were collected at a pilot slow sand filter in a long-term challenge study.

Rapid sand filtration

380 data-pairs were available with the concentration of clostridia spores before and after filtration. These data-pairs were used to calculate the removal efficiency for each sampling day. The average removal efficiency was 1.35 log. The removal data (expressed as fraction passing filtration) are plotted in the histogram in Figure 23, together with the fitted Gamma-, Beta- and Beta-Binomial distribution.

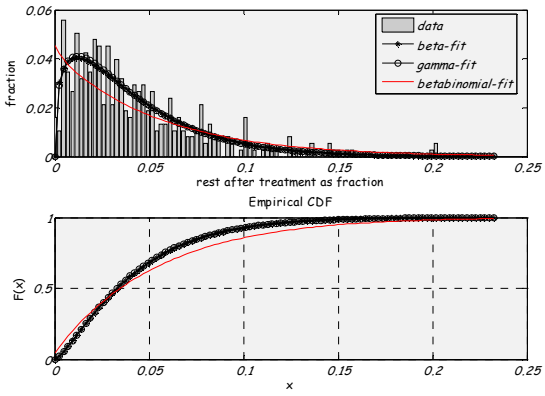


Figure 23 The fraction of spores of sulphite reducing clostridia passing rapid sand filtration and the fitted Gamma, Beta and Beta Binomial distribution.

Ozonation

For the ozonation, 355 data pairs on spores were available. 34.9% of the days that the spores were measured, no spores were detected at the outlet of the ozone reactor. For these days, no accurate removal efficiency could be calculated. However, ignoring these data would mean that the data that show a high removal efficiency would be disregarded. To be able to include these data, the zeros in the outlet were replaced by a 0.005 per litre. Two distributions were made; one of the 65.1% of the data with spores present both in the inlet and outlet and one of the 34.9% of the data with spores in the inlet and no spores in the outlet (now 0.005 per litre). Figure 24 shows the histogram of the data with spores present for in- and outlet samples with the fitted Beta and Exponential distribution and Figure 25 shows the data with spores in the inlet and no spores in the outlet with the fitted Beta distribution. The two Beta distributions (from Figure 24 and Figure 25) were selected for the QMRA, with a spore in the inlet of the ozonation having a 65.1% chance of "entering" the first Beta distribution and a 34.9% of entering the second.

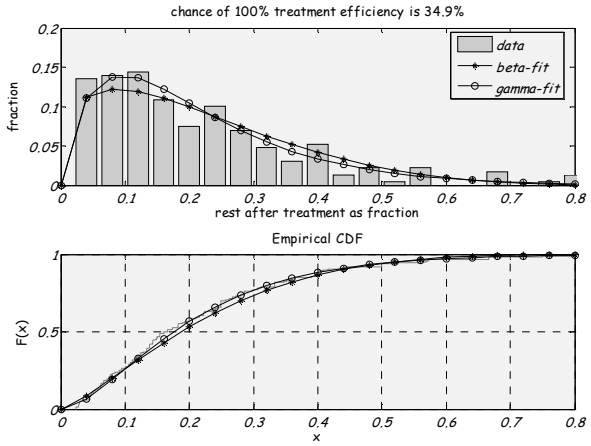


Figure 24 Frequency distribution (PDF and CDF) of the removal of spores of sulphite-reducing clostridia by rapid sand filtration (only data-pairs with spores observed in in- and outlet sample-pairs), and the fitted Beta and Gamma distribution.

Softening

The softening did not show significant removal of spores and the removal of oocysts by this process was considered 0.

GAC filtration + slow sand filtration

Data on spores were available from the inlet of the GAC filters and of the final water, after slow sand filtration, but not from the outlet of the GAC filters (=inlet slow sand filters). Hence, the data combined the removal by the two filtration processes. One hundred sixty two data points were available before GAC filtration and 2069 data points were available after slow sand filtration. However, 36% of the samples before the GAC filters contained no spores and 95% of the (1 litre) samples after slow sand filters contained no spores, so the data did not allow an accurate description of the removal by slow sand filtration. Since the slow sand filtration was considered the most important barrier against *Cryptosporidium,* it was decided to conduct a study to collect specific data on *Cryptosporidium* removal in a pilot sand filter that operated under the same conditions as the full scale filters. *Cryptosporidium* oocysts were added to the feed water over a period of three months and the oocyst concentration for in- and outlet samples were analysed. The results show a very efficient removal of oocysts equal to or more than 4.0 log [Hijnen *et al.,* in prep.].

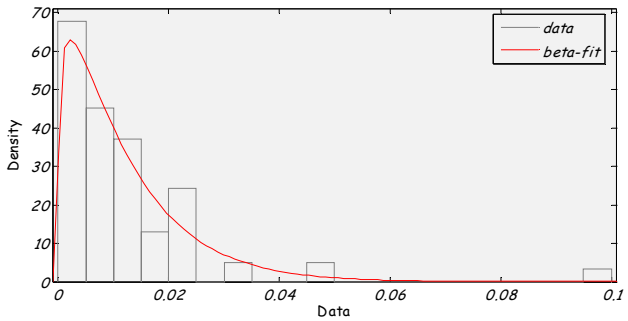


Figure 25 Frequency distribution (PDF) of the removal of spores of sulphite-reducing clostridia by rapid sand filtration (only data-pairs with no spores observed in the outlet sample), and the fitted Beta distribution.

Concentration in drinking water

Now information of the *Cryptosporidium* concentration in source water, the recovery efficiency, the removal of spores of sulphite-reducing clostridia by rapid sand filtration, ozonation and the pilot study on slow sand filtration was available and statistical distributions were fitted to each of these data-sets. The concentration of *Cryptosporidium* in drinking water was computed by taking 1,000,000 random samples from each of the statistical distributions (Monte Carlo analysis). For slow sand filtration, the removal was fixed at the average removal. Figure 26 shows the *Cryptosporidium* concentration (corrected for recovery) in source water, after rapid sand filters, after ozone, and after GAC-filtration+slow sand filtration, which is the final water that was distributed (without disinfectant residual) to the consumers. The computed median concentration of *Cryptosporidium* in drinking water was 0 per litre (95% CL: 1.8×10^{-6} per litre).

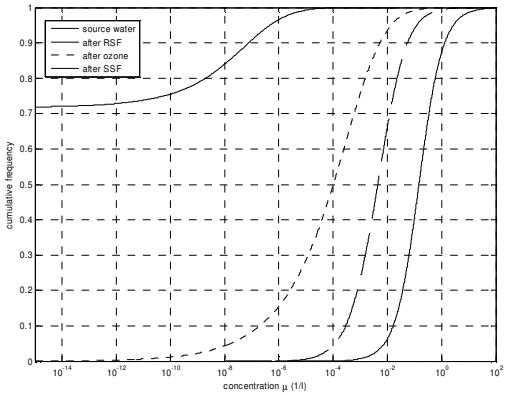


Figure 26 Frequency distribution of the concentration of Cryptosporidium (corrected for the recovery) in source water, after subsequent treatment processes and in the final treated water.

Water consumption
Following the recommendations of Mons *et al.* [2007], the conservative data on consumption of cold tap water from the Melbourne diary study of Robertson *et al.* [2000] were used. The Poisson distribution provided the best fit to these data and is relatively simple (Figure 10, Chapter 4). The Poisson distribution with a mean of 3.49 glasses (0.25 l) per day was used to assess the exposure to *Cryptosporidium* through tap water consumption.

6.6.4 Risk characterization

To characterize the risk of infection, the data on the concentration of *Cryptosporidium* in drinking water were combined with the consumption data to calculate the daily dose. These were entered in the (Iowa) dose-response relation to determine the daily risk of infection ($P_{inf,daily}$). The annual risk of infection ($P_{inf,\ annual}$) was computed by: $P_{inf,\ annual} = 1-(1-P_{inf,\ daily})^{365}$. Figure 27 shows the annual probability of exposure and the annual risk of infection. The median risk of infection is 0, with and with an upper 95%CL of 2.8 x 10^{-5} per person.

Table 11. The concentration of Cryptosporidium in source water, at several stages in the treatment and in the final treated water and the resulting probability of exposure and infection to the consumer.

Stage	Mean	P97.5
Source water	0.5607	3.7973
After RSF	0.053	0.26
After ozone	0.0083	0.032
After slow sand	1.2 x 10^{-6}	1.8 x 10^{-6}
Annual probability of exposure	8.1 x 10^{-5}	5.5 x 10^{-5}
Annual probability of infection	3.7 x 10^{-5}	2.9 x 10^{-5}

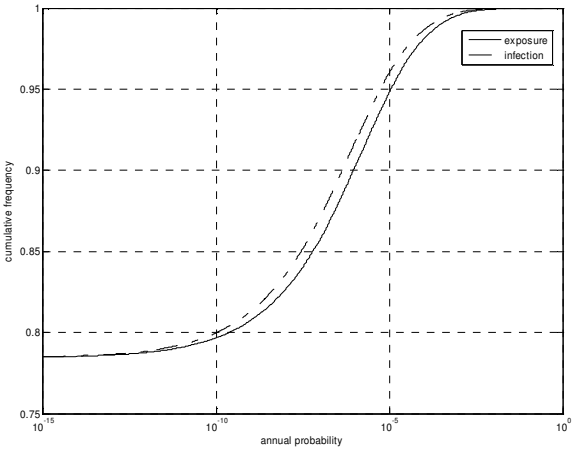


Figure 27 Frequency distribution of the probability of exposure and infection per person per day and the annual probability of infection per person.

6.6.5 Risk management

The first stage of the risk assessment, the slow sand filtration could not be adequately described (see 6.6.3). The first action was therefore to conduct the pilot plant research to collect data on *Cryptosporidium* removal by the slow sand filters.
After these data were collected the second stage of the risk assessment was conducted, which is the stage that is reported here. With the outcome of this risk assessment the water utility has demonstrated to the Drinking Water Inspectorate and to the public that their drinking water meets the Dutch guideline of 10^{-4} risk of infection with a very high certainty.

6.7 FROM HEALTH-BASED TARGETS TO TREATMENT TARGETS

In the new WHO Guidelines for Drinking Water Quality, an example is given on how QMRA can be used to transform health-based targets into treatment performance targets[3]. The health-based target for drinking water-related illness that is set in the Guidelines is a DALY disease burden of 10^{-6} per person per year [WHO, 2004]. For *Cryptosporidium*, the DALY per case is 1.5×10^{-3} [Havelaar & Melse, 2003]. This means that, in a population of

[3] The data in this paragraph are extracted from Table 7.3 of the WHO Guidelines for Drinking Water Quality [WHO, 2004].

one million people, a maximum of 666 persons may contract cryptosporidiosis via drinking water annually. If the probability of an infected person to develop clinical cryptosporidiosis is 0.7, the maximum probability of infection is 9.2×10^{-4} per person per year, or 2.5×10^{-6} per day. Using the (Iowa) dose-response relation, the maximum probability of exposure to keep below this tolerable infection risk is 6.3×10^{-4} per person per day. Assuming a consumption of 1.0 litre of cold tap water per person per day, the maximum concentration of *Cryptosporidium* in tap water is 6.3×10^{-4} per litre. The required treatment performance can now be determined from the difference in the concentration in source water and the maximum concentration in tap water (Figure 28, adapted from WHO, 2004). The infectivity of the other *C. parvum* isolates is higher than the Iowa isolate (see chapter 5). Also the probability of contracting the illness once infected is higher for the other isolates. Hence, when the combined dose-response relation of the four isolates is used and the probability of illness when infected is assumed to be 1.0, the maximum exposure to achieve 10^{-6} DALYs per person per year is significantly lower and the required treatment performance is 2.2 logs higher (Figure 28).

The health-based targets that are set may differ from country to country. The approach presented here to infer treatment performance targets from a health-based target can be used for any health outcome (probability of infection, probability of illness, DALYs etc.) that is used to set health-based targets. An advantage of this approach is that it allows a clear communication between water supplies (treatment performance) and health authorities/regulators (health target).

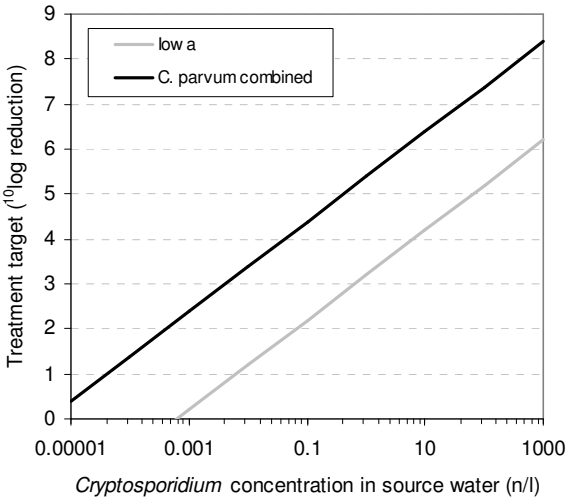


Figure 28 Treatment performance targets for Cryptosporidium parvum, based on the concentration in source water, to achieve 10^{-6} DALYs per person per year.

7

Risk management

7.1 THE VALUE OF QMRA

Water suppliers and drinking water policy makers that have to make risk management decisions are faced with information that is complex and uncertain. But risk management decisions have to and are being made anyway. Questions like: “When can I regard a water supply system as safe (enough)?”, “How much effort should be put in the protection of drinking water compared to other routes of transmission?”, “How should I weigh increased ozonation to inactivate *Cryptosporidium* to the health risk of increased bromate formation?” and many others are dealt with by risk managers throughout the world. Quantitative microbial risk assessment can help risk management in two main ways:

- QMRA provides a systematic approach basis where the best available scientific information can be combined into an assessment of the level of safety of water supply systems. A state-of-the-art QMRA of a water supply has to rely partly on assumptions. Given the current level of uncertainty in quantitative risk assessments of drinking water supply, the outcome should be regarded as an indication of the level of safety, rather than an absolute assessment of health risk. Nonetheless, the outcome can be used to guide the

risk management to the most effective ways of pathogen control and to select the most appropriate control measures, using the best available scientific basis and systematic risk assessment approach. Examples can be found in the literature (see Table 9) and are given in the case studies in Chapter 6.

- QMRA models provide a systematic combination of all relevant information and improve the understanding of the pathways and barriers involved in transmission of pathogens such as *Cryptosporidium* through drinking water. The risk assessment process also identifies important gaps or weaknesses in the available information. Gale [2002] described QMRA as a chain of mathematical calculations, in which the weakest link is often the component which is supported by poor or inadequate data with a high level of uncertainty. QMRA focuses research on the areas where improved information improves the basis for risk management most effectively.

7.2 RISK MANAGEMENT ACTIONS

Risk management requires a systems approach. The Water Safety Plan provides such an approach [Deere *et al.,* 2001; WHO, 2004; Davison *et al.,* 2005]. Risk assessment provides the risk manager with an objective indication of the need and extend of risk management measures, the Water Safety Plan is a tool to determine where and how these risk management measures will be taken and to document the safety of the drinking water system. Naturally, Drinking Water Safety Plans and the risk management actions are specific for the water supply under investigation. The above references provide a comprehensive description of the Water Safety Plan approach and examples of their implementation in water supply and the reader is referred to these for further reading.

Cryptosporidium is a pathogen that is transmitted through the faecal-oral route, like many other enteric pathogens. Many of the actions to manage the risk of *Cryptosporidium* in water supply are similar to the actions needed to control of other faecal pathogens and include protection of the catchment against contamination with faecal wastes from man and livestock, adequate water treatment processes and protection of treated water during transport and distribution to the consumers home (the multiple barrier principle). For guidance on general risk management of transmission of enteric pathogens through water supply and the multiple barrier principle, the reader is referred to the recent books of Fewtrell & Bartram [2001], Percival *et al.* [2004]; OECD/WHO [2003], Mara & Horan [2003] and Rhodes-Trussel [2005]. In this document the issues that are specific to managing the risk of *Cryptosporidium* in water supply are discussed.

Risk management actions that are most frequently employed for controlling the *Cryptosporidium* risk to water supply are:

- *Cryptosporidium* monitoring;
- enhanced catchment protection;
- optimisation of filtration in water treatment and/or

- the installation of additional water treatment processes, esp. UV and membrane filtration.

The following paragraphs give guidance on each of these actions.

7.2.1 *Cryptosporidium* monitoring

Include peak events

Monitoring for *Cryptosporidium* is, despite the drawbacks of the detection methods as discussed in Chapter 4, needed to understand the occurrence of *Cryptosporidium* in the catchment and in source waters. Most regulatory monitoring programs [ICR] and water supply monitoring programs are using sampling schemes with regular (for example monthly) intervals. Such sampling schemes may miss important peak events [Atherholt *et al.,* 1998] and it is better to guide monitoring with information about hazardous events that may occur in the catchment and lead to peak contaminations of source waters. Several authors have found a relationship between heavy rainfall and high concentrations of *Cryptosporidium* [Poulton *et al.,* 1991; Hansen & Ongerth, 1991; Stewart *et al.,* 1997; Atherholt *et al.,* 1998; Cox *et al.,* 2004]. Also sewer overflows [Gibson *et al.,* 1998] and snowmelt may lead to peaks of *Cryptosporidium* concentrations in source water. Extreme weather conditions may be a driver for peak events, both in surface and groundwater. Other causes for peak events also occur, most of them man-made, such as farming practices, accidental spills and water quantity management practices. Peak events are catchment specific; a catchment survey can identify the specific events that could lead to peaks in a specific source water. Information about the occurrence and impact of hazardous events on source water quality may be deduced from relatively simple indicators, such as rainfall, water flow, turbidity and faecal indicator bacteria [Medema *et al.,* 2001; Roser *et al.,* 2003]. Intensive monitoring for these simple and cheap indicators is helpful in guiding more complex and expensive *Cryptosporidium* monitoring programs.

Quality assurance

The shortcomings of the current detection methods for *Cryptosporidium* detection and enumeration require extensive quality assurance of the analysing laboratories. Although the methods and laboratories have improved over the years, the quality of the analysis is still very dependent on the competence of the lab personnel and quality of equipment [Schaefer, 2001]. Several countries [US, UK, Australia and others] have introduced an accreditation system for *Cryptosporidium* laboratories and proficiency testing schemes such as PHLS, LEAP, CRYPTS]. These schemes are also accessible for laboratories from other countries and it is strongly recommended that they join such schemes. Most frequent errors are the presence of false positive and false negative samples and low (or very high) recovery efficiencies.

The occurrence of false-positive samples is attributed to the presence of structures that resemble oocysts in the microscopical preparations of samples. The introduction of purification methods such as IMS and flow cytometry have reduced this background, but not completely. It is therefore important to have the ability to confirm that oocyst-like particles have the morphological features of oocysts, by using Nomarski Differential Interference

Contrast (DIC) Microscopy and/or the fluorogenic dye DAPI (4'6 diamidino-phenyl-indole) which highlights the sporozoite nuclei.
Low recoveries are the main problem of *Cryptosporidium* enumeration in water samples. It is therefore essential for analysing laboratories to include control samples to determine the recovery efficiency. In such samples, a known number of oocysts is added to a representative water sample. This seeded sample is processed as a normal sample and the number of oocysts recovered is counted. Because many studies have shown the recovery to be variable, both within and between laboratories, recovery samples should be taken by each laboratory at regular intervals. The ideal situation is to determine the recovery efficiency for each individual sample. Commercial preparations of pre-stained oocysts that can be distinguished from the "wild" oocysts in the sample are available for this purpose [Warnecke *et al.,* 2003]. Other QA samples should include method blanks and reagent (monoclonal antibodies, IMS beads) tests

Molecular typing
In recent years, there have been significant advances in the genotyping of *Cryptosporidium,* both in the available methods and the understanding of the taxonomy of the genus (see Chapter 4). Genotyping methods have been shown valuable in investigating the source of outbreaks and to verify (or falsify) the waterborne nature of outbreaks [Ong & Isaac-Renton*.,* 2003; Smith, *et al.,* 2003]. The general conclusion of a workshop on the application of molecular methods for *Cryptosporidium* monitoring [Latham *et al.,* 2003] was that use of genotyping methods will improve the risk management of cryptosporidiosis and that these methods should be employed in environmental investigations, not only retrospectively in outbreak situations but generally so that more informed handling of the results environmental surveillance is achievable. The value of molecular typing to better understand the sources of *Cryptosporidium* in the catchment and their health significance is becoming more widely recognised. Several recent studies have shown that genotyping of environmental isolates results in a better understanding of the sources of pollution of *Cryptosporidium* in source waters and their potential to cause infections in humans [Xiao, 2003, 2005; Neumann *et al.,* 2004].

7.2.2 Catchment protection

The principle aim of catchment protection is to prevent microbial contamination travel from the contamination source to the source water used for the production of drinking water as much as possible. This is an important element of the multiple barrier approach for surface water supplies, that has been employed since the Greek and Roman times [Medema *et* al 2001]. It is even more important for groundwater supplies, that usually rely an little treatment (see par. 7.2.3). Intake points from surface water supplies should be kept well away from contamination sources, such as sewer overflows, discharges of untreated and treated sewage, slaughterhouse effluents, agricultural waste disposal, sites of intensive animal farming etc. Similarly, groundwater wells should be kept well away from sewers, septic tanks, manure or sewage sludge landfills, intensive livestock farms or other storage

facilities of human or animal faeces. Even today, a simple survey of contamination sources in the vicinity of the water source may reveal the presence of sources that may threaten the water supply. An example is the Clitheroe outbreak of cryptosporidiosis [Howe *et al.,* 2002], were site inspection showed that the concrete cover of the reservoir that collected spring water as well as a wellhead cover were damaged and cattle manure was present on and around these covers. Heavy rainfall just prior to the outbreak may have facilitated transport of *Cryptosporidium* from the manure into the drinking water system. Risk management included restoring the integrity of the water abstraction systems.

Sometimes, the travel from contamination source to water intake is not directly obvious, but can still lead to outbreaks or contamination events. One example is the Milwaukee outbreak of cryptosporidiosis [MacKenzie *et al.,* 1994]; the intake of the Howard Avenue drinking water treatment plant was located in Lake Michigan, not far from the mouth of the Milwaukee River. The city's sewage treatment plant discharged in the river close to the river mouth. As the river water entered the lake, it was not mixed but created a plume of river water in the lake. The intake of the treatment plant was situated inside this plume. Initially, Spring rains and snowmelt was thought to have transported high levels of *Cryptosporidium* from the cattle farms and slaughterhouses into the harbour at the river mouth and travel to the intake. Retrospective genotyping of the outbreak isolates revealed that the outbreak was caused by *C. hominis*, the human genotype and hence originated from the human sewage rather than cattle. Chistensen *et al.* [1997] studied water quality data and mixing behaviour and found a high correlation in the turbidity of the sewage treatment effluent and the water at the intake of the drinking water treatment plant, indicating the sewage outfall was the more likely source of the outbreak. The risk management actions that were taken in the catchment were to recommend relocation of the intake farther out into the lake. Interestingly, in January 1994, a few months before the outbreak, a tunnel system was created for storage of sewerage during storm events, to prevent sewer overflows. These overflows may have contributed to previous contamination events of the Howard Avenue plant intake. The description here focuses on the catchment side of this outbreak. The outbreak was not only due to a peak contamination in the source water, but also by events in the treatment system. For an overview of the information about this outbreak (and may others), the reader is referred to the review of Hrudy & Hrudey, 2004.

Another example is given by the *Cryptosporidium* incidents in Sydney in 1998. Sydney used surface water from a semi-protected catchment with a series of dams. The final reservoir (Lake Burragorang) has a length of 55 km and a residence time of approx. 6 months. In the period of 1993-1998, the Sydney area experienced below average rainfall, and the reservoir level was low. In August 1998, heavy rain fell in the catchment. The runoff entering the lake was colder than the water in the lake. The thermal stratification in the lake caused the flood water to travel to the lake floor and towards the dam. This, together with the strong wind caused and internal wave around the thermocline. When the offtake of the water treatment plant was above the thermocline, clean long-residence time water was abstracted, but when the wave crests hit the dam wall, contaminated, short-residence time floodwater was abstracted. Detection of oocysts in the raw water coincided with these floodwater incursions [Cox, *et al.,* 2003]. The post-incident(s) risk management action were the installation of a

separate body that was responsible for the catchment management, research into contaminant sources, identifying hotspots and research into pathogen transport in the catchment. Understanding the regional processes that govern *Cryptosporidium* ingress into the surface water and the fate and transport [Davies *et al.,* 2004] helps to define the most effective risk mitigation measures.

Domestic wastewater and agricultural run-off are the main sources of *Cryptosporidium* in water. Sewer overflows and discharges are hotspots for contamination of water with *Cryptosporidium* species that are pathogenic to humans [Jiang *et al.,* 2005]. Agricultural run-off, especially from young calves and lambs is a serious risk factor for contamination of watersheds with *Cryptosporidium* that is pathogenic to humans. Since, calves and lambs of 1-4 weeks old are of highest risk of being infected with *C. parvum* [Olson *et al.,* 2004] and can shed very high numbers of oocysts, calving and lambing should be set in places where the possibility of contamination of surface or groundwater is minimised. Similarly, these young animals should be kept away from the watershed and their manure should preferably be separated and composted at high temperatures to inactivate the oocysts. If manure is applied to lands, these should be level soils and the manure should be incorporated into the soil. Open area grazing can be done with older animals, but fencing should keep them away from the direct vicinity of the watershed to prevent direct input of manure in the watershed.

7.2.3 Groundwater protection

Following an outbreak of cryptosporidiosis in North London (UK) that was traced to a groundwater supply that was vulnerable to infiltration of surface water containing *Cryptosporidium* sp. [DWI, 1998], the UK Group of Experts recommended that groundwater systems should be evaluated for potential contamination risk [Bouchier, 1998]. The current groundwater protection practice is based (as in many countries) on land surface zoning according to travel times of the water from the land surface to the groundwater sources and the restriction of contaminating activities in the most vulnerable zones. In Germany and The Netherlands, no contamination sources may be present in the zone of 60 days groundwater travel time around the abstraction. Bouchier [1998] evaluated these practices for their protective value against microbial pollution, especially with *Cryptosporidium* sp. It was concluded that this approach formed a sound basis for assessing vulnerable groundwater supplies, but that an important limitation was that by-pass features, which allow rapid transport of water with contaminants to groundwater, are not incorporated in the vulnerability assessment. By-pass flow may occur in many of the British carbonate aquifers and in other karstic aquifers. Similarly, surface water-aquifer interactions that may occur in valley-bottoms (surface water recharge) and upper catchments are not incorporated in the vulnerability assessment. Bouchier [1998] recommended the inclusion of an additional vulnerability class in the zoning scheme. This extreme vulnerability class would apply to areas with the combination of contaminated surface water and rapid access points (solution features, sinkholes, karst or pseudo karst features, mines and aggregate extraction sites).

The need for the inclusion of rapid access of surface water to groundwater as an important factor in vulnerability assessment was illustrated by the fact that eight of the nine suspected cases of groundwater contamination with *Cryptosporidium* sp. in the UK were associated with adited wells, collectors, spring galleries and former mines with adits [Morris and Foster, 2000]. Groundwater supplies in rural settings were more commonly affected than sites in urban settings. Fissure flow, dual porosity flow and intergranular flow were all represented in these cases. Intergranular flow appeared only to be important in settings where the residence time in the aquifer was very short, such as in river gravels close to a surface water course.

The Expert Group listed the factors of a groundwater system that need to be considered for assessing the risk of contamination with *Cryptosporidium* sp. (Table 12) and gave guidance on techniques to determine/verify the significance of these factors. Simple qualitative ranking may help to prioritise the different hazards, however, Morris and Foster [2000] stress the need to focus on the individual water supply when applying ranking, appreciating the unique hydro-geological, operational and contamination sources setting of each supply system. Special attention should be given to the risk of contamination during rain events. Heavy rains may facilitate rapid transport of oocysts from the sources through the groundwater or through cracks in the abstraction system (see description of the Clithero outbreak in par. 7.2.2). Many contamination incidents and outbreaks are associated with heavy rainfall [Curriero *et al.,* 2001].

Table 12 Factors for consideration in the risk assessment of groundwater contamination (adapted from Bouchier, 1998)

Predisposing groundwater to *Cryptosporidium* sp. Risk	Possible verification techniques
Catchment factors	
High wastewater returns, including sewage effluent to river reaches, especially under baseflow conditions	Hydrochemistry, microbiology, hydrometry
Livestock rearing in inner catchment, especially if intensive	Farm survey
Likely *Cryptosporidium* sp. - generating activities in catchment – e.g. abattoirs	Economic activity survey
Urbanising catchment	Land registry survey
Livestock grazed or housed near wellhead	Site inspection
Hydrogeological factors	
Known or suspected river aquifer connection nearby	Flow gauging, modelling, hydrochemistry
Unconfined conditions with shallow water table	Well-water level monitoring
Karst or known rapid macro-fissure flow conditions, especially in shallow groundwater	Field mapping, farm survey
Patchy drift cover associated with highly contrasting aquifer intrinsic vulnerabilities	Field mapping, shallow drilling
Solution features observed or inferred in catchment	Field mapping
Shallow flow cycles to springs	Tracing, hydrochemistry, water temperature logging
Fissure-dominant flow (as suggested by high transmissivity or specific capacity)	Downhole fluid/flow logging, pumping test analysis
Well/raw water source factors	
Supply source tapping shallow flow systems (e.g. adits, springs, mine galleries)	Check site plans, tracing

Adits with upbores or construction-stage ventilation shafts	Check site plans, site inspection
Poor casing integrity	CCTV, geophysical logging
Masonry linings above pumping water level without additional sanitary seal	CCTV, check site plans
Sewer/septic tank/slurry pit systems near wellhead or above adits	Site inspection
Inadequately fenced source especially around spring boxes, catchpits, galleries	Site inspection
Old, poorly documented well construction	Site plans/National well record archive

7.2.4 Optimised particle removal by water treatment

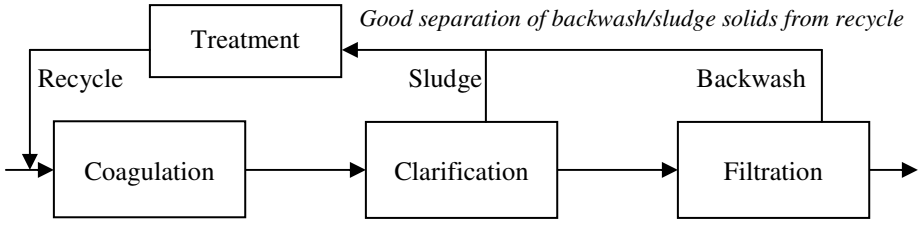
Many of the waterborne outbreaks have been associated with treatment inadequacies [Bouchier, 1998; Hrudey & Hrudey, 2004]. Several outbreaks might have been prevented if the treatment systems were operated in accordance with good practice. This means that maintaining good practice, monitoring the performance of treatment systems and taking corrective measures when monitoring indicates treatment is compromised (see Water Safety Plan) is essential.

Many important recommendations for risk management of *Cryptosporidium* in water treatment are already given by the Group of Experts in the UK [Bouchier, 1998]. These are directed primarily at conventional water treatment, which is coagulation/floc removal/filtration. The recommendations of the Group of Experts are given in

Effective oocyst capture ***Good solid/liquid separation*** ***Ripening period***
Readily separable floc ***Breakthrough***

Figure 29 . The most important point of attention for oocyst removal by conventional treatment [from Hall *et al.,* 2000].

Box 1 (slightly adapted). The highlights are presented in Figure 29. During a filter-run, the first time (hour) after backwashing represents a major risk of oocyst breakthrough. Risk management actions may be filter-to-waste or slow start of filters after backwashing. Towards the end of the filter-run, particle removal is reduced and filters need to be backwashed. Particle counting of the effluent of individual filter is a too to detect increased particle breakthrough as early as possible [Edwards, 2000; Mazounie *et al.,* 2000] and helps to optimise filter performance.



Effective oocyst capture ***Good solid/liquid separation*** ***Ripening period***
Readily separable floc ***Breakthrough***

Figure 29 . The most important point of attention for oocyst removal by conventional treatment [from Hall *et al.,* 2000].

Box 1. Recommendations for optimised conventional water treatment (adapted from Bouchier, 1998).

Water treatment works should be designed to handle the typical peak turbidity in the source water.

Water treatment works should be operated at all times in a manner that minimises turbidity in the final water; attention should also be given to other parameters which reflect the performance of chemical coagulation, that is, coagulant, metal concentration, and colour.

Water treatment works should normally be operated within the design capacity and without by-passing of the solids-liquid separation processes which are responsible for removal of turbidity and coagulant solids; coagulation itself should never be by-passed or compromised.

In the event of an emergency, if it is necessary to overload or by-pass solid-liquid separation processes, a stringent monitoring regimen should be initiated to ensure that turbidity targets of 0.1 NTU are not exceeded. If there is an indication that these targets will not be achieved, an immediate advice to boil notice should be issued.

For high risk sites, if minimisation of the effects of filter start-up on final water quality cannot be achieved through more easily implemented changes (for example improved backwash or delayed start after backwash), modifications to the works should be made to allow the first flush to be run to waste or recycled to the works inlet.

Coagulation/flocculation processes should be checked regularly to meet changing conditions of source water quality and other environmental factors

Only dedicated washwater mains should be used to carry the returned washwater flow.

Filters should be operated and maintained under optimum conditions with attention to the quality and depth of media and to the operation of the backwashing/air scouring system.

Treatment works staff should be trained to be aware of the potential effect on the final water quality of even very small changes in the catchment or the treatment stream.

Water utilities should check that process monitoring systems are appropriate to the risk at each source. For high risk sites, monitoring should include continuous turbidity measurement at the outlet of each filter and on the final water using instruments capable of detecting changes of less than 0.1 NTU.

Appropirate action procedures to react immediately to turbidity alarms should be in place; actions might include immediate isolation of the filter, or, if suggested by history, the issue of advice to boil.

7.2.5 Additional treatment

Membrane filtration
Additional treatment processes for *Cryptosporidium* control are primarily membrane filtration and UV disinfection. Before 1998, the focus was on membrane filtration as initial studies on UV inactivation of *Cryptosporidium,* using in vitro viability assays, had indicated that UV is not effective against *Cryptosporidium* [Lorenzo-Lorenzo *et al*., 1993; Ransome, Whitmore & Carrington, 1993; Campbell *et al.* 1995] (see Chapter 4). Different types of membrane systems LeChevallier & Au [2004] give an overview of the efficacy of microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) against *Cryptosporidium* and other pathogens and indicator organisms. In general, membrane filtration can remove *Cryptosporidium* very effectively; reported log-removals are typically in the order of 5 – 7 logs, with higher removals also reported. If membranes are installed primarily for *Cryptosporidium* control, microfiltration is usually the system of choice, due to the relatively low costs and ease of operation. MF does not remove viruses, so if a broader barrier against pathogens is necessary, the finer pore size of UF is required. RO and NF are usually not selected primarily as pathogen barrier but for desalination, softening and/or micropollutant control.
In some cases, significantly lower removals are seen in pilot or full scale studies. These low removals are attributed to the composition and integrity of the membranes. Integrity of the membrane systems (membrane material, seals, spacers, membrane housing etc.) is crucial if these high log removals need to be maintained. Several integrity tests have been developed that can be used when the system is off-line, such as the pressure-hold test and bubble point test. Monitoring system integrity on-line is even more important. At the UF/RO water treatment plant of Heemskerk (The Netherlands) counting of (0.05 µm and larger) particles is used to monitor the UF system and sulphate monitoring is used to monitor the integrity of the RO system on-line [Kruithof *et al.,* 2001].

UV
After 1998, when *Cryptosporidium parvum* was found to be very sensitive to UV [Clancy *et al.,* 1998], UV became a widely studied additional treatment system for *Cryptosporidium* control. Several water utilities in the world are installing or have already installed UV for the control of pathogens such as *Cryptosporidium* (Seattle; Rotterdam; New York); several of these on the basis of QMRA [van der Veer, 2002; LeChevallier, 2004]. UV is effective against most enteric pathogens [Hijnen *et al.,* 2005]. Low and medium pressure lamps appear to be equally effective against *Cryptosporidium.* Recently, Johnson *et al.* [2005] demonstrated that *C. hominis* is as sensitive to UV as *C. parvum.* Compared to other enteric pathogens, *Cryptosporidium* is more sensitive to UV than viruses, but less sensitive

compared to most bacteria. Rapid inactivation of oocysts is readily observed at low UV doses (3 logs at 5 – 10 mJ/cm^2 [Hijnen *et al.,* 2005]. At higher UV doses, Craik *et al.* [2001] observed considerable tailing in the inactivation data. The cause for this tailing is still under debate. If it is a biological phenomenon, this would limit the efficacy of UV to 3 log inactivation of *Cryptosporidium.* Repair of UV damage, as reported for bacteria, is present in oocysts; Morita *et al*. [2002] demonstrated photo-reactivation and dark-repair of DNA in *Cryptosporidium parvum* with the endonuclease-sensitivity site assay. The animal infectivity, however, was not restored. Similar observations were reported by Shin *et al.* (2001) and Zimmer *et al.* (2003).

Qian *et al*. [2004] combined the available data on UV inactivation of *Cryptosporidium* with a statistical method (Bayesian meta-analysis) which resulted in the relation between UV dose and *Cryptosporidium* inactivation presented in Figure 30 [USEPA, 2003]. Due to the uncertainty over the inactivation at higher doses, this relation was calculated for an inactivation up to 3 log. The relation is described by the following formula:

$$Log\left(\frac{C_{out}}{C_{in}}\right) = 1.2344\ln(UVdose) - 0.1283$$

With C_{in} and C_{out} being the concentration of infective *Cryptosporidium* oocysts at the in- and outlet of the UV reactor.

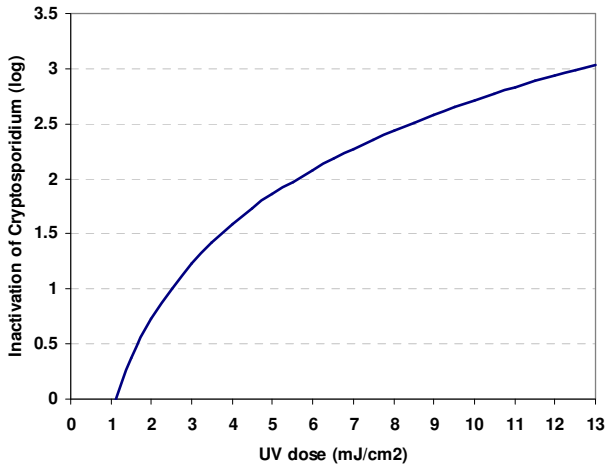


Figure 30 Relation between Cryptosporidium inactivation and UV dose [after USEPA, 2003]

An important issue in the application of UV in water treatment is the assessment of the net UV dose in UV reactors in treatment practice. The UV *intensity* is usually monitored with one or more UV sensors in the reactor (preferably at the reactor wall), but the UV *dose* depends on the distribution of the UV intensity and the water flow in the UV reactor chamber. Reactor design and hydraulics are very important as even a small fraction of short-

circuiting seriously limits the disinfection efficacy. Standards and guidelines have been developed to determine the effective (or Reduction Equivalent) dose of UV systems [DVGW, 1997; ONORM, 1999; USEPA, 2003]. In these guidelines, the reduction equivalent UV dose is determined by challenging the UV system with a micro-organism with well-characterised UV inactivation kinetics (such as MS2 phages or *Bacillus subtilis* spores). The inactivation of the challenge organism that is observed when operating the UV system under a range of test conditions (water flow, lamp power, UV transmission of feed water) is used to calculate the reduction equivalent UV dose at each setting. When a calibrated sensor is used, the sensor reading can be employed during operation of the UV system in practice to monitor the performance of the UV system over time.
Other factors that are important for the operation of UV systems are the ageing of lamps, fouling of lamp sleeves and sensors, and the presence of particles in the feed water, since they may shield pathogens from UV.
More background on the assessment of the net UV dose and the factors that influence the efficacy of UV systems can be found in the EPA Guidelines [USEPA, 2003] and in Sommer *et al.,* 1997; 1998; 2000.

7.2.6 Distribution

Although not typically an area where specific *Cryptosporidium* control measures are used, the protection of the integrity of the distribution network is especially important against *Cryptosporidium. Cryptosporidium* is very resistant to chlorine, so the residual chlorine levels that are used to protect the distribution network against the consequences of ingress of contaminants do not work against *Cryptosporidium.* The same applies to groundwater systems that do not use filtration or UV.
The barriers in the distribution network are the integrity of the system, the water pressure and backflow prevention in the connections of the network to the domestic plumbing installations. Any breach in these barriers may result in a contamination. Hence, low pressure events, leakage, cross-connections and mains breaks are indicators of the presence of a health risk, even in the presence of a disinfectant residual. Strict hygiene during construction, maintenance and repair of distribution networks and groundwater abstraction systems are equally important control measures to prevent *Cryptosporidium* (and other pathogens) to enter drinking water.
Comprehensive guidance on proper operation, maintenance and risk management of the distribution network can be found in a recent WHO review document by Ainsworth *et al.* [2004].

7.3 RESEARCH PRIORITIES

7.3.1 Exposure assessment

Detection methodology
The detection and enumeration methods for *Cryptosporidium* in environmental samples still need considerable improvement. The main areas are increased and consistent recovery efficiency, confirmation of presumptive oocysts and the ability to determine the viability and infectivity. Genotyping methods are evolving rapidly and should be implemented in environmental surveys to increase our understanding of the environmental transmission of human cyptosporidiosis.

Catchment and source water protection

Treatment
A large amount of data have been collected on the removal of *Cryptosporidium* during filtration or the inactivation upon exposure to disinfectants. Most of these studies are done in laboratory experiments or pilot scale treatment systems. This information can be and is used in QMRA-studies in the form of generic log-credits (see Case study 1 and 2). This is very useful in screening-level QMRAs to set risk management priorities. As the screening-level QMRA has indicated that a water supply system may be at risk, a more site-specific QMRA is needed. In the vast majority of water supplies, no site-specific data on the removal of *Cryptosporidium* are present. To extrapolate the body of available scientific knowledge and water treatment experience to a treatment process at a specific site, performance models for the removal of *Cryptosporidium* by the treatment process are very useful. This is already used in ozone disinfection for instance, using the disinfectant concentration, contact time, reactor hydraulics and temperature as model parameters [ESWTR, Smeets *et al.,* 2006].
The performance of a treatment process is not constant. As a result of variation in feed water quality, temperature, water flow and process control, the removal of pathogens varies over time [Gale *et al.,* 2002; Hijnen *et al.,* 2000; 2004; LeChevallier & Au, 2004]. Of particular importance for QMRA are the moments of poor performance [Gale, 2002]. In a well-operated treatment process, these moments are scarce, but when they occur, they result in a relatively high health risk. Many of the outbreaks are related to treatment deficiencies. Data on the occurrence of such events are scarcely available in the scientific literature, but are present in the logs of water utilities [Westrell *et al.,* 2003] and may be deduced from treatment performance monitoring data (i.e. turbidity, particle counts, disinfectant residual) or the microbiological monitoring programs (*E. coli* and other microbial indicators) of water utilities.
When microbial indicators (spores of sulphite-reducing clostridia, aerobic spores) are used to establish the removal of *Cryptosporidium* by a specific full-scale system on-site, it is important to know if and how the removal of the indicator correlates with the removal of *Cryptosporidium.* This can be established in studies where the removal of *Cryptosporidium*

is compared to the removal of the indicators under identical conditions [Emelko *et al.,* 2003; Chung *et al*, 2004].

UV is increasingly used for *Cryptosporidium* control in water treatment. Several issues around UV inactivation of *Cryptosporidium* warrant further investigation. The 'tailing' of the inactivation kinetics at higher UV doses potentially limits the efficacy of UV, while the high sensitivity of oocysts observed at low doses suggests that high log-inactivations are achievable. Suggested cause of this phenomenon is a resistant subpopulation of oocysts, but also experimental artefacts may result in these observations. Understanding of the cause of this apparently reduced sensitivity is important to determine the scope of UV as barrier against *Cryptosporidium* in water treatment. The occurrence of at least part of the cellular mechanism to repair the damage of UV to the oocysts DNA appears to be inconsistent with the observation of several authors that no restoration of infectivity occurs after exposure to low doses of UV. A better understanding of the UV repair capacity of oocysts is needed.
A practical problem of the current EPA guidelines for the testing of UV reactors specifically with the objective to assign log-credits for *Cryptosporidium* is that the biodosimeters that are currently used are much more resistant to UV. The pattern of inactivation over the reactor is therefore different for *Cryptosporidium* and the biodosimeters. A bias-factor is introduced in the EPA-guidance manual, but an alternative biodosimeter with a UV sensitivity similar to that of oocysts would be more reliable.

Distribution
To be able to assess the risk of the ingress of material in the distribution network during low or no pressure events, leaks in the network, e.g. in reservoir covers or during maintenance and repair, data are needed on:
- the occurrence (frequency, magnitude) of contamination events;
- the presence of *Cryptosporidium* in the material that may enter the network during these events.

A QMRA that attempted to calculate the risk of contamination events in the distribution network have used the water utilities logs to collect data on the frequency of such events and has used assumptions on the type (sewerage) and amount of material that entered the distributed water [Westrell *et al.,* 2003] during these events. The use of the microbiological monitoring programmes of distributed water may provide information about the frequency and also the magnitude of contamination events. This is now under investigation.

7.3.2 Effect assessment

Cryptosporidium parvum is, at the moment, the only pathogenic micro-organism for which there is information on the natural variation in infectivity and pathogenicity in human hosts. Four different isolates have been studied and appeared to have different dose response properties (Chapter 5). Two different groups of human subjects have been studied in their responses to the same type of oocysts and here too, there appeared to be substantial variation

in dose response relations. Still, there remains much to be investigated. All oocyst types studied so far belonged to *C. parvum*, infectious to humans and to other mammals. A feeding study with *C. hominis*, which exclusively infects humans, has just been reported [Chappell *et al.,* 2006]. The ID_{50} was 10-83 oocysts, in the low range of the *C. parvum* strains. Interestingly, there appears to be a better dose-illness and dose-IgG respons relation for *C. hominis.* Subtyping of the *C. parvum* isolates found in human infections is indicating that some subtypes are more frequently, maybe even exclusively, found in humans [Smith, pers. comm.]. Employing genotyping methods will increase our understanding of the taxonomy and of the transmission pathways of *Cryptosporidium.*

The older QMRA literature uses the dose-response of the Iowa isolate. The exponential dose-response relation that was inferred from the human volunteer data predicts a probability of infection at a dose of a single oocyst of 0.42%. The data on the TAMU isolate yield a probability of infection by ingestion of a single oocyst of 5.9% [Deere & Davison, 2004]. For the UCP and Moredun isolate this is around 40%. The combination of the dose-response function of the four isolates as presented in Chapter 5 gives a probability of infection of 20% for ingestion of a single oocyst. This means that the QMRA in the older literature may have underestimated the risk. If more isolates were tested in human volunteer studies, this percentage is likely to change further and complying with an acceptable risk level may prove a moving target. However, a simple approximation could be to assume that the infectivity is 100%. This would mean that the probability of infection equals the probability of exposure. This will overestimate the risk by a factor of 2.5 for the UCP and Moredun isolate and 5 for the combined isolates, which is relatively small compared to other factors (see Chapter 6).

A somewhat more technical problem is related to the detection of oocysts excreted by infected subjects. Faecal detection is problematic and the immunofluorescence method is known to be too insensitive to detect low numbers of excreted oocysts. The performance or recovery of the detection method is accessible to independent measurement. Given a certain recovery, the statistical analysis of dose response data could be corrected for false negatives.

Hazard identification does not stop with the estimation of individual risks of infection or acute illness. Translation to the population level is not completely trivial, and involves new problems: sampling effects in small (sub-)populations, temporal variation, nonlinear relation between involved numbers and costs, and the impact of secondary infection. A pathogen like *Cryptosporidium* that is transmitted through the faecal—oral pathway cannot be assumed to spread homogenously in a host population. The potential for secondary spreading by direct contact is probably high in certain environments (within households, day-care centres, schools, …) and low everywhere else. Therefore, a transmission model must account for different compartments, with high transmission in small subpopulations. For these small compartments, stochastic effects (due to small numbers of subjects) cannot be neglected.

Dose response assessment is a problem area because required information is hard to find and studies specifically targeted at obtaining such information (human feeding studies) are

usually very expensive. Therefore, the information on dose response relations of microbial pathogens in humans will always be limited. Even for *Cryptosporidium*, where an impressive data base has been built in the last decennium, there are many essential aspects of hazard characterization that remain unclear. Therefore, a common approach for assessing the risk of a pathogen when such dose response data are missing, is the use of a surrogate.
Several problems are associated with such a choice: the surrogate organism has some properties in common with the true pathogen, but similarities are based on observable biological characteristics, and not on similar infectivity or pathogenicity. Recent developments in molecular typing methods have greatly increased the amount of detail with which a micro-organism can be characterized. However, the properties of interest here concern the *interaction* between pathogen and host, for which the coincidence of certain specific factors in both host and pathogen is necessary, often accompanied by certain environmental conditions. Therefore, even when virulence factors in a pathogenic micro—organism can be identified, their presence in a different microorganism under different conditions need not tell us very much about infectivity/pathogenicity of this latter organism.
On the other hand information from various sources – the presence of certain genetic markers in pathogen and/or host, outbreak investigations where pathogen type and amount ingested have been recorded, vaccine studies— adds to the knowledge of the dose response relation in some way or another. Modern statistical techniques are being developed to deal with such information from various sources and of varying quality.

7.3.3 QMRA versus epidemiology

Whenever a risk model has been set up in enough detail to have some uncertainty estimates, it would be highly interesting to try and confront the estimated risks with epidemiological data. Although this involves many additional uncertainties that cannot always be easily quantified, it is the closest we can get to a validation with 'real world' data. By choosing a scenario and calculating incidences (or prevalences, in a transmission model) by means of Monte Carlo procedures, the agreement between the risk model and observed outbreak data could be quantified. A waterborne outbreak with well-characterised health and water quality data would be the best scenario for such a comparison. Haas *et al.* compared the epidemiological investigations of the Milwaukee outbreak with a QMRA on the *Cryptosporidium* concentrations that were found in samples of ice that was prepared from mains water at the time of the contamination and concluded that the results of the QMRA are consistent with the epidemiological investigations (see par. 6.2). Confirmation of this relation by comparing QMRA and epidemiology at other outbreaks (preferably good water quality data) would strengthen the use of QMRA as a health risk assessment tool.

8
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