REVIEW ARTICLE

# Virus hazards from food, water and other contaminated environments

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## Abstract

Numerous viruses of human or animal origin can spread in the environment and infect people via water and food, mostly through ingestion and occasionally through skin contact. These viruses are released into the environment by various routes including water run-offs and aerosols. Furthermore, zoonotic viruses may infect humans exposed to contaminated surface waters. Foodstuffs of animal origin can be contaminated, and their consumption may cause human infection if the viruses are not inactivated during food processing. Molecular epidemiology and surveillance of environmental samples are necessary to elucidate the public health hazards associated with exposure to environmental viruses. Whereas monitoring of viral nucleic acids by PCR methods is relatively straightforward and well documented, detection of infectious virus particles is technically more demanding and not always possible (e.g. human norovirus or hepatitis E virus). The human pathogenic viruses that are most relevant in this context are nonenveloped and belong to the families of the *Caliciviridae*, *Adenoviridae*, *Hepeviridae*, *Picornaviridae* and *Reoviridae*. Sampling methods and strategies, first-choice detection methods and evaluation criteria are reviewed.

## Introduction: main food and environmental virus hazards

Food and environmental virology mostly studies viruses that can be transmitted through water, sewage, soil, air, fomites (objects capable of transmitting microbial pathogens) or food (Bidawid *et al.*, 2009). Most such viruses are enteric viruses transmitted via the faecal–oral route. Infected humans can excrete large amounts of human pathogenic viruses; animal and plant material as well as other excreta and secreta can also carry high viral loads (Breitbart *et al.*, 2003; Zhang *et al.*, 2006; de Roda Husman & Bartram, 2008). Viruses transmitted via the faecal–oral route are generally nonenveloped and thus very stable in the environment (Rzeżutka & Cook, 2004) and include major aetiological agents, some of which are thought to be emerging zoonotic pathogens. These viruses cannot always be effectively eliminated by current meth-





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ods of sewage treatment (Vantarakis & Papapetropoulou, 1999; Thompson *et al.*, 2003; Van Heerden *et al.*, 2003; Van den Berg *et al.*, 2005) and consequently cause viral contamination of the environment from treated as well as untreated wastewater. Other examples of indirect routes are run-off from manure used in agriculture. There is also direct faecal contamination of the environment from humans and animals, for example by bathers or by defecation of free-range or wild animals onto soil or surface waters. The resulting viral contamination of sea and coastal water, rivers and other surface waters, groundwaters, and irrigated vegetables and fruit is associated with subsequent risks of reintroduction of the viral pathogens into human and animal populations (Yates *et al.*, 1985; Metcalf *et al.*, 1995; Muscillo *et al.*, 1997; Koopmans *et al.*, 2002; La Rosa *et al.*, 2007). Human exposure to even low levels of these pathogenic viruses in the environment, such as norovirus (NoV), can cause infection and disease (Lindesmith *et al.*, 2003; Teunis *et al.*, 2008). Individuals with an impaired immune system, including children, the elderly, pregnant women and people with HIV/AIDS, are more susceptible to such infections, and the disease outcome may be more severe. This is the case, for example, for rotavirus (RV), which is a more serious problem for young children in developing than in developed countries (Havelaar & Melse, 2003). Genetic susceptibility may also play a role in the susceptibility to infection, as in the case of NoV and the ABO histo-blood group receptor genotype (Hutson *et al.*, 2002).

Environmentally transmitted viruses include major aetiological agents of mild diseases such as gastroenteritis as well as agents of more severe diseases such as meningitis and hepatitis. Most of these viruses belong to the families *Adenoviridae*, *Caliciviridae*, *Hepeviridae Picornaviridae* and *Reoviridae* (Dubois *et al.*, 1997; Muscillo *et al.*, 2001; Lodder & de Roda Husman, 2005). The major enteric virus families include one or several types and variants of virus; the different groups may differ as concerns persistence, pathogenicity and infectivity. Some of these viruses, such as hepatitis E virus (HEV) (the sole member of the *Hepeviridae*), are thought to be zoonotic pathogens. New human pathogenic viruses that may also be transmitted via the environment emerge frequently (McKinney *et al.*, 2006). Enteric viruses are predominantly transmitted via the faecal–oral route and are present in wastewater; therefore, such water is a potential source of infection if not treated or used appropriately (Gantzer *et al.*, 1998; Baggi *et al.*, 2001; Asano & Cotruvo, 2004). These agents are adapted to the hostile environment of the gut and in most cases, can persist for a very long time in water, soil or food matrices (Raphael *et al.*, 1985; Richards, 2001; Le Cann *et al.*, 2004; Van Zyl *et al.*, 2006; Espinosa *et al.*, 2008; Hansman *et al.*, 2008).

## Caliciviruses: major viral causes of gastroenteritis

NoV and sapovirus (SaV) are the most important human agents of diarrhoea worldwide (Patel *et al.*, 2009). NoVs are the leading cause of food-borne outbreaks of acute gastroenteritis and the most common cause of sporadic infectious gastroenteritis affecting people of all age group (Green, 2007; Patel *et al.*, 2008, 2009). SaVs are mainly associated with sporadic acute gastroenteritis in young children (Hansman *et al.*, 2007a; Khamrin *et al.*, 2007; Monica *et al.*, 2007) and are less commonly involved than NoV in epidemic gastroenteritis (Green, 2007), although some outbreaks have been described (Johansson *et al.*, 2005; Hansman *et al.*, 2007b, c). The burden of calicivirus (including NoV) has been clearly documented in numerous geographical areas worldwide (Hall *et al.*, 2005; EFSA, 2009; Scallan *et al.*, 2011).

NoVs and SaVs are icosaedric nonenveloped viruses with an ssRNA (+) genome of between 7.3 and 8.3 kb. They are both classified within the family of the *Caliciviridae*, as the genera *Norovirus* and *Sapovirus*, each subdivided into five genogroups (Karst *et al.*, 2003) and several serotypes. Three genogroups (GI, GII and GIV) containing more than 20 genotypes of NoV are known to infect human beings, and the intra-genotype nucleotide diversity can be as high as 15% (Zheng *et al.*, 2006). Most human infections are caused by GI and GII, whereas GIII affects swine. In the case of SaV, at least four distinct genogroups containing a number of genotypes and variants can infect humans (Farkas *et al.*, 2004). Thus, NoV and SaV detection can be difficult owing to the large number of genogroups and genotypes; furthermore, currently available detection methods are not sufficiently powerful, and indeed, the prevalence of uncommon NoV variants is probably underestimated (La Rosa *et al.*, 2008).

NoV is believed to be transmitted mainly by person-to-person contact or by aerosols after projectile vomiting (Marks *et al.*, 2000, 2003). Consumption of food or water contaminated by faecal matter or vomitus (Marks *et al.*, 2000, 2003; Rutjes *et al.*, 2006), and exposure to contaminated surfaces or fomites, are also the sources of infection (Wu *et al.*, 2005; D'Souza *et al.*, 2006). The ease with which NoV is transmitted and spread is mainly because of its infectious dose being low – fewer than 10 virus particles are required for the infection (Teunis *et al.*, 2008) – high resistance to disinfection (Duizer *et al.*, 2004a; Jimenez & Chiang, 2006; Whitehead & McCue, 2009) and possible long-term stability and persistence in the environment (Wu *et al.*, 2005; D'Souza *et al.*, 2006).

The most common cause of NoV food-borne outbreaks is the consumption of shellfish, fresh produce and ready-

to-eat food contaminated by infected, but possibly asymptomatic, food handlers (Daniels *et al.*, 2000; Cannon & Vinjé, 2008; Lamhoujeb *et al.*, 2008). The long-term stability and persistence of NoV on contaminated surfaces used in food preparation areas also make a substantial contribution to disease transmission (Cheesbrough *et al.*, 2000; Evans *et al.*, 2002; Kuusi *et al.*, 2002; Taku *et al.*, 2002; Clay *et al.*, 2006; D'Souza *et al.*, 2006; Mattison *et al.*, 2007; Lamhoujeb *et al.*, 2008, 2009). Moreover, NoV is resistant to many industrial food preservation methods and can survive chilling, freezing, acidification, reduced water activity and modified atmosphere packaging (Baert *et al.*, 2009).

NoV has also been documented as a water-borne pathogen, and numerous outbreaks have originated from sewage-polluted drinking water (Nygård *et al.*, 2003; Maunula *et al.*, 2005; Hewitt *et al.*, 2007; ter Waarbeek *et al.*, 2010) and recreational water (Hoebe *et al.*, 2004; Maunula *et al.*, 2004; Sartorius *et al.*, 2007). This may be a consequence of its suspected resistance to wastewater treatment (Lodder & de Roda Husman, 2005; Van den Berg *et al.*, 2005; da Silva *et al.*, 2007; La Rosa *et al.*, 2009; Nordgren *et al.*, 2009; Skraber *et al.*, 2009) in addition to its survival ability in aquatic settings (Kadoi & Kadoi, 2001; Allwood *et al.*, 2003; Bae & Schwab, 2008). Additionally, shellfish grown and harvested in wastewater-polluted water can concentrate NoV, which may be inadequately eliminated by standard depuration procedures (Muniain-Mujika *et al.*, 2002): the consequence is outbreaks of gastroenteritis after consumption of shellfish (Le Guyader *et al.*, 2006a; Le Guyader *et al.*, 2008; Webby *et al.*, 2007).

## Hepatitis A virus: prevalent in developing countries

Hepatitis A virus (HAV) is an icosaedric nonenveloped virus species with an ssRNA (+) genome of approximately 7.5 kb and is classified in the family of the *Picornaviridae*, genus *Hepatovirus*. Approximately 1.4 million people worldwide become infected with HAV annually (Issa & Mourad, 2001). The incidence of infection varies between regions of the world, with the highest rate in developing countries where sewage treatment and hygiene practices can be poor. Conversely, the number of reported cases of HAV infection has declined substantially in countries with effective programmes of immunization with a licensed vaccine. For example, in the USA, the number of cases has been reduced by 92% to an infection rate as low as one case per 100 000 persons per year (Daniels *et al.*, 2009); similar situations now also apply to other countries including Canada, Australia, Japan and New Zealand (Jacobsen & Koopman, 2004).

HAV can, via sewage discharge, contaminate soil, food crops and natural watercourses (Bosch, 1998; Cook & Rzeżutka, 2006). Consequently, food (Pebody *et al.*, 1998; Hutin *et al.*, 1999; Lees, 2000; Dentinger *et al.*, 2001; Nygård *et al.*, 2001; Greening, 2006) and drinking water (Divizia *et al.*, 2004; Tallon *et al.*, 2008) are considered major vehicles of HAV transmission to humans. In an epidemiological investigation, 6.5% of acute cases of hepatitis A were identified as food- or water-borne; however, this figure is probably an underestimate, because a considerable proportion of cases (~68%) remain uncharacterized (Daniels *et al.*, 2009).

HAV is able to survive in several environments, notably in water, food and soil (Rzeżutka & Cook, 2004). Water is considered to be the most important source of infectious virus because it can survive for long periods in this environment. For example, the virus can survive for up to 60 days in tap water (Enriquez *et al.*, 1995), over 6 weeks in river water (Springthorpe *et al.*, 1993), over 8 weeks in groundwater (Sobsey *et al.*, 1989) and even up to 30 weeks in sea water (Crance *et al.*, 1998). HAV is also able to survive in various types of soil and remains infectious after 12 weeks (Sobsey *et al.*, 1989).

## Adenoviruses: some serotypes cause gastroenteritis in children

Adenovirus (AdV) is an icosaedric nonenveloped virus with a dsDNA genome 28–45 kb long. They are classified as members of the *Adenoviridae* family, genus *Mastadenovirus,* which includes 20 known species: three bovine, five human and three porcine. Fifty-one serotypes of human AdV (hAdV) in six subgroups (A-F) have been described (Wold & Horwitz, 2007). hAdV serotypes 40/41, included in Group F, are the major causes of gastroenteritis in young children and are readily spread by the faecal–oral route. They are sensitive to chemical disinfection but are more resistant to the effects of UV light than other enteric viruses (Thurston-Enriquez *et al.*, 2003). hAdV is shed from the gut on a long-term basis regardless of the site of initial infection, although the mechanism has not been fully clarified in humans (Calcedo *et al.*, 2009; Echavarria, 2009; Roy *et al.*, 2009). A limited number of probable water-borne outbreaks of hAdV have been reported, particularly in association with conjunctivitis and swimming pools (Martone *et al.*, 1980). Chlorination failures are often cited as a major factor in outbreaks.

## Enteroviruses: common viral causes of gastroenteritis

The genus *Enterovirus* (EV) comprises spherical nonenveloped viruses, with an ssRNA (+) genome of 7.2–8.5 kb,

in the family of the *Picornaviridae.* Four species have been distinguished (A, B, C and D) within which the serotypes are known by their traditional names: human EV (hEV) A includes some coxsackievirus A strains; hEV B contains coxsackievirus A9, coxsackievirus B1-6 and most of the echoviruses; and hEV C contains polioviruses 1–3 and some coxsackievirus A strains. The more recently identified hEVs have been given individual numbers, from EV68, and are classified amongst all four species (Stanway *et al.*, 2005).

These viruses may replicate in the respiratory tract and the gut and can be transmitted through aerosols and by the respiratory route or via the faecal–oral route. Many infections are asymptomatic, and as few as one in 100 may result in clinical illness. The wide range of diseases includes classical poliomyelitis, aseptic meningitis, cardiac disease, hand, foot-and-mouth disease, conjunctivitis and rashes. A common clinical picture is self-limiting fever, malaise, muscle aches and headache; diarrhoea and vomiting are present only as a part of more generalized systemic illness. Clinical illness in temperate climates is more common in the summer months; all age groups are affected, and immunity to one serotype does not protect against infection with other serotypes (Moore *et al.*, 1984). The serotypes of echoviruses and coxsackieviruses then circulate and dominate within communities change over time, and there is molecular drift within serotypes (Savolainen *et al.*, 2001). hEVs can be found in all aquatic matrices reflecting their widespread occurrence in populations (Sellwood *et al.*, 1981; Hovi *et al.*, 1996; Sedmark *et al.*, 2003). However, transmission of hEV infection through an aquatic route has been difficult to confirm as the number of asymptomatic infections is so large and the transmission by close personal contact so common.

## HEV: zoonotic transmission as an emerging problem

HEV is a small, spherical and nonenveloped ssRNA (+) virus of approximately 7.2 kb. It is classified within the family of the *Hepeviridae*, genus *Hepevirus.* HEV is a major cause of acute human hepatitis in regions with inadequate water supplies and poor sanitary conditions (Purcell & Emerson, 2001; Guthmann *et al.*, 2006), and there is an increasing evidence of locally acquired HEV infections in industrialized countries (Zanetti *et al.*, 1999; Widdowson *et al.*, 2003; Buti *et al.*, 2004; Mansuy *et al.*, 2004; Ijaz *et al.*, 2005; Waar *et al.*, 2005). HEV sequences worldwide can be classified into four major genotypes (1–4) (Lu *et al.*, 2006). The relatively conserved genotypes 1 and 2 circulate primarily in humans causing the majority of HEV infections including all epidemics in Asia and Africa countries and also in Mexico. By contrast, for genotypes 3 and 4, only isolated cases of human infection have been described and only in more industrialized countries including the USA, Japan, China and countries in Europe. Although four genotypes of HEV exist, there only seems to be one serotype present (Zhou *et al.*, 2003; Herremans *et al.*, 2007; Mushahwar, 2008). Previously, HEV infections in industrialized countries were believed to be travel related, but recently an increasing number of indigenous HEV cases have been reported (Zanetti *et al.*, 1999; Widdowson *et al.*, 2003; Mansuy *et al.*, 2004; Lu *et al.*, 2006; Borgen *et al.*, 2008). Serological studies have reported the presence of HEV antibodies in a variety of animal species, notably cows, cats, dogs and rodents. However, HEV RNA has not been detected in these species, and the validity of the assays used is seldom well established owing to the lack of positive reference samples: consequently, these results must be interpreted with caution (Bouwknegt *et al.*, 2007). The presence of HEV has been reported in food, water and animals including pigs (Rutjes *et al.*, 2009a). In several animal species, HEV genotype 3 and 4 sequences have been detected, with pigs being the animal most frequently involved in countries formerly designated as nonendemic for HEV. HEV RNA has also been detected in wild boar in several countries (Takahashi *et al.*, 2004; de Deus *et al.*, 2008; Martelli *et al.*, 2008; Adlhoch *et al.*, 2009), in Sika deer (Tei *et al.*, 2003), in roe deer (Reuter *et al.*, 2009), in red deer (Rutjes *et al.*, 2010) and in mongoose (Nakamura *et al.*, 2006). Furthermore, a human HEV genotype 1 strain was detected in workhorses in Egypt (Saad *et al.*, 2007).

The non-travel-related HEV infections in industrialized countries may be of zoonotic origin. Sequences of the swine HEV genotype 3 and 4 strains closely related to human strains have been isolated in many countries worldwide (van der Poel *et al.*, 2001; Huang *et al.*, 2002; Clemente-Casares *et al.*, 2003; Lu *et al.*, 2006; Rutjes *et al.*, 2007; Reuter *et al.*, 2009), suggesting that pigs may be the reservoir of the indigenous infections in these countries. More direct evidence of zoonotic food-borne transmission of genotype 3 was obtained when four cases of hepatitis E could be linked directly to eating raw deer meat: identical HEV strains were found in the deer meat consumed and the patients (Tei *et al.*, 2003; Li *et al.*, 2005).

## RV, astrovirus and other agents of gastroenteritis: water-borne pathogens affecting mostly children

Viruses of the genus *Rotavirus* are icosahedral nonenveloped nonturreted virions with a triple capsid structure and a segmented dsRNA genome of approximately

18.5 kb. They are classified in the *Reoviridae* family, and there are five major groups (A-E) (Estes & Kapikian, 2007). Group A RV (GARV) is associated with a large majority of human RV infections and represents the major cause of child mortality because of diarrhoea worldwide (Parashar *et al.*, 2006; Sánchez-Padilla *et al.*, 2009). GARV is also widespread in wild and domestic animal species, and it has been suggested that zoonotic transmission plays a substantial role in the introduction of novel strains into the human population (Cook *et al.*, 2004; Bányai *et al.*, 2009). Within GARV, at least 19 G- and 27 P-types can be distinguished on the basis of sequence diversity of the genes encoding the two outer capsid proteins (VP7 and VP4) (Matthijnssens *et al.*, 2008; Van Doorn *et al.*, 2009). The recent introduction of vaccines for human use may lead to the emergence of novel RV genotypes or the re-emergence of older strains, particularly from animal reservoirs, and such strains could displace those currently predominating (Cook *et al.*, 2004; Iturriza-Gómara *et al.*, 2004; Kang *et al.*, 2005; Steyer *et al.*, 2008).

RV persist similarly in polluted and nonpolluted fresh water (Hurst & Gerba, 1980) and even when subjected to light exposure, which can seriously affect the stability and viability of other enteric RNA viruses, for example astrovirus (Fujioka & Yoneyama, 2002; Lytle & Sagripanti, 2005). Inactivation of virus infectivity in different types of water has been consistently found to correlate with higher temperatures (John & Rose, 2005).

The genus *Mamastrovirus* (AstV) includes spherical nonenveloped viruses with an ssRNA (+) genome of between 6.8 and 7 kb. They are members of the *Astroviridae* family. There are six species affecting bovines, felines, mink, ovines, porcines and humans (HAstV). HAstV is a common cause of gastroenteritis in children and also in the elderly and immunocompromised individuals (Herrmann *et al.*, 1991; Guix *et al.*, 2002; Mendez & Arias, 2007). Eight genotypes of HAstVs have been described to date and are classified into genogroup A (HAstV-1 to 5 and HAstV-8) and genogroup B (HAstV-6 and 7) (Gabbay *et al.*, 2007). HAstVs have been occasionally found associated with gastroenteritis outbreaks involving possible water-borne or food-borne transmission (Leclerc *et al.*, 2002; Maunula *et al.*, 2004; Smith *et al.*, 2006; Domínguez *et al.*, 2008; Scarcella *et al.*, 2009), and their presence in seafood has been discussed and may depend on rainfall conditions (Le Cann *et al.*, 2004; Riou *et al.*, 2007). Recently, the possible zoonotic transmission of astroviruses from cows was proposed (Kapoor *et al.*, 2009).

Other viruses, such as kobuvirus, aichivirus, picobirnavirus and torovirus, are also found in the environment, but further epidemiological studies and wide-ranging investigations of diagnostic spectra are needed to document their distribution in the environment and impact on food safety and health.

## Shedding of pathogenic viruses into the environment

### Zoonotic transmission

One of the main routes of transmission of viruses to humans is zoonotic, associated with the consumption of contaminated products of animal origin, or during food manipulation by infected handlers. The other most frequent cause of virus-contaminated foods is contact with faecal-polluted waters (Fig. 1). Inadequately treated drinking water, consumption of crops contaminated after being irrigated with wastewater or fertilized with sewage and ingestion of shellfish grown in polluted waters are, therefore, common causes of food-borne viral infection of people (Bosch, 1998). Several factors affect the contamination of shellfish, vegetables, berries, fruits and herbs. Climatic variables such as season, tidal cycles, rainfall and flooding have all been implicated in viral contamination of the environment (Le Guyader *et al.*, 2000; Griffin *et al.*, 2003; Suffredini *et al.*, 2008; Guillois-Bécel *et al.*, 2009). Likewise, good livestock, agriculture and manufacturing practices are absolutely necessary to minimize the risk of viral contamination of food. Inappropriate irrigation practices, wastewater treatment and reuse, sewage overflows, and wastewater releases from polluted sources are the direct causes of viral environmental contamination and food-borne outbreaks (Le Guyader *et al.*, 2000; Griffin *et al.*, 2003; Jiménez-Clavero *et al.*, 2003; Choi *et al.*, 2004; Suffredini *et al.*, 2008; Guillois-Bécel *et al.*, 2009) (Fig. 1). Shellfish grown in areas close to intensive farming, or waste treatment plants, present a high risk of enteric virus carriage (Le Guyader *et al.*, 2000; Ley *et al.*, 2002).

There has been increasing concern about the effects on human and animal health of pathogenic viruses in animal manure. In recent years, outbreaks of food-borne diseases associated with the consumption of animal products have received much attention, leading to consumer concern about the safety of the food supply. The health risk associated with animal operations depends on diverse factors. The most important is related to the animal species being reared and the concentration of pathogenic microorganisms in animal manure. Some viruses survive both for long periods and despite treatment, and their ability to remain infectious in the environment until ingested by a human or animal host is an added concern. However, it has been difficult to determine the role of livestock in most water-borne virus outbreaks because both humans and various wildlife species can shed the

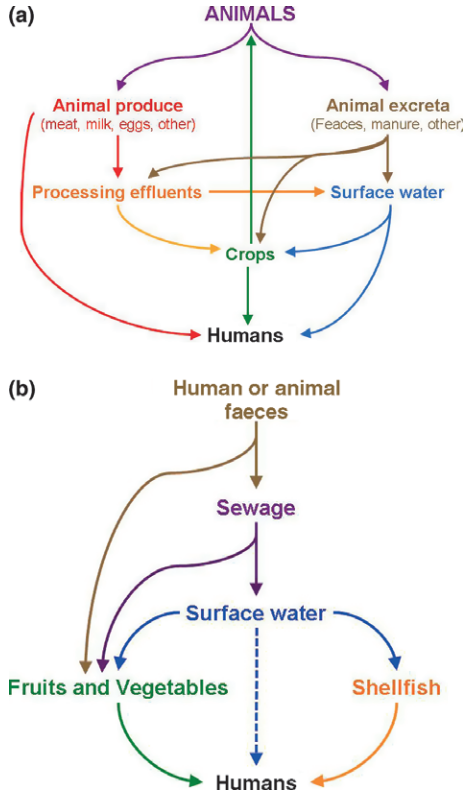


**Fig. 1.** Contamination routes for environmental virus hazards (a) of animal origin and (b) in foods. (a) Contamination routes of environmental virus hazards of animal origin. Zoonotic route of contamination from the original source (animal) to humans. (b) Environmental virus contamination of foods. Contamination from original source to humans using food and water as a route of transmission.

same viruses and thereby serve as sources of infection or contamination. EVs are shed in faeces and, consequently, are disseminated through contaminated soil and water; therefore, any other animal species grazing in the same pastures and/or drinking from the same water sources as infected livestock are likely to be exposed. Consequently, they may be contaminated by the same or closely related virus variants and therefore present a high risk of further disseminating the virus (Ley *et al.*, 2002; Jiménez-Clavero *et al.*, 2005).

Most pathogenic viruses emerging in human populations are of animal origin (Taylor *et al.*, 2001). There is a large spectrum of transmission modes for zoonotic viruses with domestic animal or wildlife reservoirs. They can be direct or indirect (Kruse *et al.*, 2004) and include transmission by contaminated food, water, air and soil (Fig. 1). Meat can be contaminated by excreta during processing, but may also have been contaminated earlier because of infection of the living animal. The risk of food-borne infection depends on the virus infection route, the level of contamination and the extent of inactivation during food processing. Livestock industries produce large amounts of residues that can cause substantial environmental problems. Indeed, accidental or deliberate spills, overuse of fertilizer and emissions of incorrectly, or incompletely, treated animal wastes are the major environmental risks (Jongbloed & Lenis, 1998; Jiménez-Clavero *et al.*, 2005). Cook *et al.* (2004) estimated that contamination of arable land with animal RV in spread animal waste used as fertilizer may be considerable, and similarly substantial contamination is plausible or even likely for other viruses shed in large numbers in animal excreta. As expected, detection of animal viruses in contaminated waters (groundwater, lakes, rivers, estuaries, runoffs and animal watering tanks from farms, etc.) is much more frequent in areas of intensive than less active farming (Jiménez-Clavero *et al.*, 2005). The modes and the levels of environmental contamination with viruses differ for the different types of viruses and animal species.

## Occupational exposure

The working environment and procedures can be sources of viral dissemination. However, the difficulties associated with evidencing cases and relating them to possible exposure make it very complex to assess the risk of infection. Health care facilities are the most extensively studied occupational settings. In such facilities, blood-borne viruses, including human immunodeficiency virus (HIV), hepatitis B virus and hepatitis C virus, can be transmitted mainly by accidents with infected needles or sharp objects (Davanzo *et al.*, 2008). Air-borne viruses such as the influenza virus, respiratory syncytial virus, AdV, rhinovirus, coronavirus, measles, rubella, mumps viruses and parvovirus B19 are also easily spread (Aitken & Jeffries, 2001). Viral agents transmitted via the faecal–oral route, such as RV, hAdV 40 and 41 and NoV, are frequently associated with nosocomial and health care–related infections spread by contamination of air, hands and surfaces (Lopman *et al.*, 2004). Workers involved in sewage treatment and reuse for agricultural and industrial purposes can be exposed to enteric viruses. Seroepidemiological surveys show that workers in wastewater treatment plants (Clark *et al.*, 1985; Heng *et al.*, 1994; De Serres & Laliberte, 1997; Weldon *et al.*, 2000; Divizia *et al.*, 2008) and in spray irrigation activities (Katzenelson *et al.*, 1976; WHO, 2006) are at higher risk than the general population, in terms of enteric and hepatic infections. Veterinary and zootechnical jobs can also expose workers to zoonotic viruses through contact with manure and inhalation of aerosols generated by activities such as washing and cleaning (Cook *et al.*, 2004). Serological studies indicate that workers in the intensive animal husbandry sector

may be exposed to zoonotic viruses, notably H1 swine influenza virus (Olsen *et al.*, 2002). Workers in these fields of activity may therefore possibly have a role in species-jumping from animal to human populations (Baker & Gray, 2009).

## Environmental matrices containing human pathogenic viruses

Human pathogenic viruses are excreted and secreted by humans into their environment through faeces, urine, saliva, sweat and tears (de Roda Husman & Bartram, 2008). The principal matrices, which can be contaminated with human viruses and represent potential sources of infection, are water, sewage, sludge, manure, air, hard surfaces, crops such as fruit and vegetables, shellfish and animal products. The range of complexity in the structure and electrostatic charge of these matrices and of the viruses is such that their interactions are extremely diverse, with corresponding differences as concerns virus inactivation and removal. In general, virus survival is influenced by parameters such as moisture, temperature, association with solids and exposure to UV.

### Water and sewage

Surface waters can readily become contaminated with viruses. In the EU, guidelines for sewage discharge (Directive 91/271/EEC) concerning urban wastewater treatment were adopted in 1991 to protect the water environment from the adverse effects of discharges of urban wastewater and from certain industrial discharges. This is an important standard as it not only regulates the conditions of discharge according to the inhabitant equivalent but also stipulates requirements for corresponding collection and treatment facilities. However, the reduction values required for discharges from urban wastewater treatment plants are evaluated according to chemical and biochemical parameters, including biochemical oxygen demand, chemical oxygen demand, total suspended solids and total phosphorus and nitrogen; they do not address highly stable pathogens, like viruses. In sludge (solids remaining after wastewater treatment), viruses may be present and constitute a potential hazard.

Drinking water is abstracted from surface water in many countries and treated by sedimentation, filtration and/or disinfection, which, if done effectively, can produce a virus-free end product, although this may be dependent on the quality of the source water (Rutjes *et al.*, 2009b; Teunis *et al.*, 2009; Lodder *et al.*, 2010). The European Directive concerning quality of water intended for human consumption is Directive 98/83/EC. Monitoring should provide information about the organoleptic and microbiological quality of the water supplied as well as information concerning the effectiveness of drinking water treatment (particularly disinfection). This directive includes microbiological limits based on bacterial standards, but viruses are not considered in any of the current directives.

### Manure

Manure can be defined as urine and faecal material produced by animals housed in artificial environments, such as farms and zoos. It may also contain straw bedding, is often stored for long periods and is used as a fertilizer on agricultural land. In general, enteric viruses including caliciviruses, HAV and HEV are considered to be stable in faeces (Rzeżutka & Cook, 2004). After dispersion of viruses into the environment, the inactivation rates differ substantially between types of virus and inactivation is faster in liquid manure (mixture of urine and water with less bedding material) than in solid manure. Enteric viruses can survive for a very long time (even years) at temperatures below 5 °C and especially in the absence of UV light. There is good evidence that inactivation of viruses in the environment is less effective if they are absorbed onto or embedded within suspended solid matter that is not dried out. Viruses like HAV, NoV and HEV can resist complete inactivation in the environment for a very long time (Pesaro *et al.*, 1995).

### Air and hard surfaces

The importance of air-borne spreading of enteric viruses is not well defined, unlike water-borne or food-borne spreading. This is largely owing to the difficulties in identifying this transmission route for single cases or outbreaks. The air-borne transmission of viruses is dependent on the likelihood of material containing viruses to form aerosols and on the survival of viruses in the air. Enteric viruses can be aerosolized by, for example, violent vomiting (as associated with NoV) (Marks *et al.*, 2000), toilet flushing (Barker & Jones, 2005), spray irrigation (Petterson *et al.*, 2001) and various processes at wastewater treatment plants (Carducci *et al.*, 1995, 2000). Some enteric viruses can cause infection by ocular contact or by inhalation and virus catchment by mucus and subsequent swallowing. Nevertheless, the most common mechanism of dissemination is the deposition of aerosol particles on surfaces, particularly food, vegetation and clothes. Surfaces such as door handles, banisters for staircases, flushing handles on toilets, toys, telephones, drinking cups and fabrics have all been implicated in the transmission of enteric viruses (Barker & Jones, 2005; Gallimore *et al.*, 2008). Faecal material or vomit may contaminate these

surfaces, and the viruses contained may then be ingested following direct contact or transfer from hands (Boone & Gerba, 2007). The characteristics of the material and the virus contribute to determining the survival rate (Abad *et al.*, 1994; Vasickova *et al.*, 2010). The detection of virus on a large variety of surfaces, like tables, door knobs, walls, toilets seats, thermometers, toys, cotton cloth, carpets, bed covers, gloves, drinking glasses, paper (Boone & Gerba, 2007) has helped to explain the routes of transmission of NoV (Wu *et al.*, 2005; Boxman *et al.*, 2009a), RV (Ansari *et al.*, 1988) and rhinovirus (Ansari *et al.*, 1991) in localized cases and outbreaks.

## Food

Food and food environments are a major source of viral transmission to humans (Koopmans *et al.*, 2002; Koopmans & Duizer, 2004). Food-borne viral outbreaks are reported worldwide every year and are associated with a wide variety of foods (e.g. Verhoef *et al.*, 2008; Kuo *et al.*, 2009; Robesyn *et al.*, 2009; Vivancos *et al.*, 2009). The viruses most frequently involved in food-borne infections are NoV and HAV, but other viruses, particularly human RV, hEV, HEV and AstV, are also transmitted by food. For NoV and HAV, person-to-person spread is the most common transmission route. Secondary spread of these viruses after introduction by, for example, food-borne contamination is common and often results in larger, prolonged outbreaks (WHO and FAO, 2008). Estimates of the proportion of viral illnesses attributed to food are in the range of around 5% for HAV to 12–47% for NoV. However, all currently available estimates of food-borne illnesses make assumptions and use extrapolations from different data sources (Scallan *et al.*, 2011). Nevertheless, all essentially conclude that viruses are an important cause of food-borne illness (WHO and FAO, 2008; Scallan *et al.*, 2011). The incidence of outbreaks of food-borne viral disease has increased considerably during the last decades, possibly due to the rapid globalization of the food market, the increase in personal travel and food transportation, and the profound changes in food consumption habits (Rodríguez-Lázaro *et al.*, 2009).

Food products can be contaminated at various points along the food supply chain. This can be because of poor practice in primary production and/or misuse of natural and environmental resources (Appleton, 2000), e.g. the irrigation of vegetables with polluted water – including contamination through roots owing to drop irrigation (Urbanucci *et al.*, 2009) – contact with human faeces or faecally soiled materials and poor hygiene practice by food handlers during the harvest of fresh produce. Furthermore, contamination may arise by inappropriate practices during processing or at the point of sale/consumption (Boxman *et al.*, 2009b). Also, there may be cross-contamination from polluted working instruments or surfaces, which have been contaminated previously by infected food handlers or contaminated food items (D'Souza *et al.*, 2006; Boxman *et al.*, 2009b; Dreyfuss, 2009). In addition, shellfish, fresh produce or ready-to-eat foods may be contaminated with human excreta, either directly or indirectly, and viral food-borne outbreaks may also originate from zoonotic viruses intrinsically present in food consumed. This has been demonstrated for HEV in raw meat and liver from wild boar and deer (Matsuda *et al.*, 2003; Tei *et al.*, 2003; Takahashi *et al.*, 2004). Moreover, the potential for food-borne transmission is a concern with every new emerging infection, even for viruses that are primarily respiratory, for example, the highly pathogenic avian influenza virus. Indeed, infectious avian influenza virus has been cultured from frozen exported meat, raising the issue of possible dissemination of such viruses via the food chain (WHO and FAO, 2008).

Foods commonly implicated in outbreaks are those that are minimally processed, such as shellfish or fresh produce, although ready-to-eat foods that have been contaminated by an infected food handler are also involved. Traditionally, bivalve mollusc shellfish such as oysters, mussels, clams and cockles have been considered as a principal source of food-borne virus that may subsequently be disseminated (Pintó *et al.*, 2009). Filter-feeding shellfish can concentrate viruses from polluted water: the filtration can lead to concentrations in shellfish 100–1000 times higher than that in the surrounding water (Carter, 2005). In addition, specific binding of NoV to the shellfish epithelia has been observed, and this may impede the release of virus during shellfish depuration (Le Guyader *et al.*, 2006b; Maalouf *et al.*, 2011). Fresh produce has high water content – absorbed from groundwater during growth – and may be eaten raw and without peeling, both procedures that may remove external contamination. Viruses can survive on their surface once harvested (Carter, 2005) and can remain infectious for several days or weeks and even during commercial and household storage for periods of up to 5 weeks (Bosch *et al.*, 2006). However, any food that has been manipulated by food-handlers and is not (or insufficiently) subjected to subsequent preservation and/or cooking is susceptible to be a source of transmission of enteric viruses.

Virus survival in foods can be affected by diverse factors. Kott & Fishelson (1974) found that poliovirus persisted longer on tomato and lettuce plants in phosphate-buffered saline than in oxidation pond effluent, possibly due to microbial activity in such effluents. Also, natural irradiation in combination with natural antiviral substances generally present in fruit may greatly reduce

virus infectivity (Konowalchuk & Speirs, 1978). However, natural or added constitutes in food such as fat, salt and sucrose may protect viruses against inactivation by heating or high hydrostatic pressure (Kovač *et al.*, 2010). Conversely, components like acids and various components of fruit juices may enhance the rate of viral inactivation (Kovač *et al.*, 2010).

## Sampling strategies

### Surveillance of food and environmental virus hazards

For successful public health intervention regarding food and environmental virus hazards, the early and accurate identification of infectious viral agents is of primary importance. The ability to identify quickly the causative viral pathogen of an emerging viral epidemic markedly increases the chances of success of any countermeasures for containment, prevention and control of the possible disease. Surveillance of environmental viruses can underpin the detection of both cases and outbreaks by identifying an increase in frequency of disease above its background incidence (Centers for Disease Control and Prevention, 2001) and by estimating disease impact. In addition, surveillance can help generate hypotheses and stimulate research, evaluating prevention and control measures and facilitating planning.

Many countries and international organizations, notably the World Health Organization (WHO) and the European Centre for Disease Prevention and Control (ECDC), and international research projects have devoted considerable energy to developing integrated surveillance networks; these networks are for tracking environmental viruses including food- and water-borne viral pathogens such as NoV, RV and EV and for providing information about the viruses' genetic structure and geographical distribution and about the host populations and environmental matrix involved. Recent advances in molecular biology, including DNA chip technology and whole-genome sequencing technologies, continuously improve diagnostic power to detect and characterize a wide range of pathogens and their variants. Public health surveillance systems for outbreak detection can establish the relative value of different approaches for the detection of outbreaks at the earliest stages and provide the information needed to improve their efficacy. However, substantial costs can be incurred in developing, enhancing and managing these surveillance systems and investigating false alarms (Wagner *et al.*, 2001). Furthermore, the overall economic benefits of surveillance systems for early detection and response to outbreaks have not been clearly established.

### Sampling methods

A rational sampling plan is essential for the analysis of human pathogenic viruses, which may be present in small quantities and distributed heterogeneously in matrices; the plan should be established on a risk-based approach (Andrews & Hammack, 2003; Food Standard Agency, 2004a, b). Consequently, a sample or subsamples must represent the original matrix (e.g. water and food), and the sampling process (including the storage and transportation) must not alter the condition of the sample and thus not affect the subsequent analysis (Food Standard Agency, 2004a, b). Other aspects that also must be considered when developing a sampling programme are the characteristics of the matrix to be analysed (nature: solid, semi-solid, viscous or liquid; type: food, water or environmental sample; composition: rich in fat, protein or plant contents such as tannins; and amount: scarce or abundant), and the subsequent analytical method to be used (cell culture, immunological or molecular). If, for example, a sampling plan for a pâté factory is required, a balanced approach needs to be based on the observation that a sample suitable for public health (for example 25 g of a pâté) might not be suitable for subsequent analysis using a molecular method because of the heterogeneous nature and composition of the matrix. Any inadequacy concerning one of the aspects will affect the validity of the final analytical result.

Various international bodies, such as the International Organisation for Standardisation (ISO), the European Committee for the Normalisation (CEN) and the European Food Safety Authority (EFSA), and national bodies, such as the U.S. Department of Health and Human Services (USDHHS), have defined principles and/or standards for the sampling of foods and water. For example, ISO has established a series of standards for sampling (ISO 5667 series, ISO 18593:2004; ISO 8066:2004; ISO 24276:2006; ISO 7002:1986; ISO 17604:2003); however, there is no specific mention of sampling for human enteric pathogenic viruses in any of these standards. The CEN/ISO *ad hoc* expert committee for viruses in food 'CEN/TC 275/WG6/TAG4' is currently working on the first international standard for a horizontal method for the detection of HAV and NoV in food. However, the sampling process is not included in this planned standard, and the committee has decided to examine the ISO 6887 series for suitability. Similarly, the FDA's Bacteriological Analytical Manual (BAM) includes a general protocol for '*food sampling and preparation of sample homogenate*' (Andrews & Hammack, 2003), in which the scientific basis for sampling only uses previously published bacteriological criteria (ICMSF, 1986, 2002),

despite the BAM having defined a specific protocol for the detection and quantification of HAV (Goswami, 2001).

A large number of studies are related to viral food- and water-borne outbreaks, sporadic cases or studies using samples collected to determine the presence of different enteric viruses in food or the environment or to evaluate new methods for the detection of viruses in diverse matrices (Supporting Information, Tables S1 and S2). Several important lessons can be learnt from these studies. First, there is an evident lack of harmonization in the sample size, and therefore, a serious risk in the representativeness of the sampling strategies used. This is most important as most of those studies are related to viral diarrhoeal outbreaks: the consequences may include the true aetiological agent of the gastroenteritis not being found, or the infectious dose being under- or overestimated. In these studies, sizes of samples used were extremely diverse, ranging from 50 μL to 3000 L (i.e. an almost $10^8$-fold difference) for water and from 1.5–200 g for food samples. Second, there is a lack of homogeneity in the selection of the animal tissues or part of the sample tested once the sample is collected. This also can affect the detection of human pathogenic viruses. For example, different shellfish tissues can be tested for human enteric viruses (i.e. the whole shellfish, the mantle, the gills, the stomach or the digestive diverticula). However, it has been demonstrated that the efficiency of recovery can differ substantially between types of sample and even that the virus may not be detectable in some (Wang *et al.*, 2008). In a study evaluating different tissues of naturally contaminated oysters to identify the most suitable for the detecting virus, the percentages of samples positive were different for the whole oyster (0.7%), mantle (2.2%), gills (14.7%), stomach (13.9%) and the digestive diverticula (13.2%), and the detection was not possible when the adductor muscles were tested (Wang *et al.*, 2008). Another important factor is the ambiguous use of individual or pooled samples for foodstuffs, especially in the case of shellfish. This affects directly both the representativeness and analytical sensitivity of the final results. For example, de Roda Husman *et al.* (2007) observed that pooling digestive glands of several oysters never resulted in a positive signal, whereas RT-PCR testing of the individual digestive glands of single oysters revealed the presence of virus RNA. This indicates that pooling can affect the final results negatively and even can produce false negative results owing to the simple mechanism of reducing the size of each individual sample used in the pool. This can be of great relevance to public health. Conversely, the use of individual samples can also affect the representativeness of the population studied. A balanced approach to difficult food matrices may therefore be to analyse a representative number of individual samples; however, this could greatly increase both the cost and the time required for the analyses and even may be unfeasible in the field. Two other important aspects also have to be considered: the period of time from the sampling to the start of the analysis in the laboratory and the conditions of storage of the sample during that period. These issues can be of particular importance if complex matrixes are analysed, as the stability of the virus may be compromised. However, they are usually not rigorously addressed during sampling, and most studies do not provide the relevant details. Even where this information is provided, the lack of uniformity is again evident. Samples are sometimes stored frozen (Loisy *et al.*, 2000; Schvoerer *et al.*, 2000, 2001; Donaldson *et al.*, 2002), refrigerated at 4 °C (Pina *et al.*, 2001; La Rosa *et al.*, 2007), at room temperature (Beuret *et al.*, 2002) or kept on ice (Noble & Fuhrman, 2001; Katayama *et al.*, 2008).

## Sample representativeness

Representativeness expresses the degree to which sample data accurately and precisely reflect a characteristic or variable at a sampling point. Representativeness is a qualitative factor, which is largely dependent on the appropriate design of the sampling programme. The representativeness criterion is best satisfied by making certain that sampling locations are selected suitably and that a sufficient number of samples are collected. The sampling strategy must be unbiased, sufficient (i.e. it summarizes all relevant information about the parent population, which contained the sample, but ignoring any sample-specific information), efficient (i.e. the more the statistical values for various samples cluster around the true value and the lower the sampling error, the greater the efficiency) and consistent (the larger the sample, the closer the statistic should be to its true value) (Jarman, 1984).

## Transport and storage

After sampling is completed, samples should to be transported to the laboratory facilities as soon as possible. For example, the AFNOR method XP T 90-451 '*Recherche des entérovirus*' in water (AFNOR, 1990) states that after *in situ* concentration by filtration, the sample cartridge should be removed and enclosed aseptically such that the filtration device must not be left completely dry; thereafter, samples should be transported to the laboratory within 24 h at a suitable temperature. On the other hand, the ISO method 19458 '*Water quality – Sampling for microbiological analysis*' (ISO, 2006), although not specific for mammalian virus, states that viruses should be transported and stored for a period of 24–72 h, at a tempera-

ture of 5 ± 3 °C. The guidelines '*Standard Methods for the examination of Water and Wastewater*' (Eaton *et al.*, 2005) states that samples cannot be held more than 2 h at temperatures of 25 °C or 48 h at 2–10 °C; samples have to be stored at −70 °C if not processed in this time frame. Dahling & Wright (1984) also indicate that samples stored at −70 °C are stable without virus loss for up to 4 days. Mocé i Llivina (2004) tested the stability of EV at −70 °C and demonstrated that they could infect cells after 11 months of storage at this temperature when adsorbed to cellulose ester membranes. In conclusion, transport and storage should be performed as quickly as possible, at a controlled temperature (5 ± 3 °C). In this temperature range, samples can be stored for up to 48 h. If this time cannot be respected, the samples should be frozen at −70 °C.

It is of utmost importance that laboratory personnel recognize that the safe and efficient transportation of any infectious substance is in the interest of public health generally. The packaging of infectious substances for transport must therefore be designed to minimize the risk of damage during transport. Sending or transporting infectious viruses should respect the '*Guidance on regulations for the Transport of Infectious Substances 2009–2010*' (WHO, 2008). Different forms of transportation (road, rail, sea and air) of infectious substances have different safety requirements and therefore their own international convention or code based on UN Model Regulations. As far as laboratory personnel are concerned, their responsibility lies in ensuring that the goods are packaged according to WHO regulations. Some countries have their own national regulations; when this is not the case, International Guidelines should be followed.

### Safety in the laboratory

HAV and NoV are both classed as Hazard Group 2, with a vaccine currently being available for HAV. HEV is classed as Hazard Group 3 in some countries, and therefore, any intentional use of this virus in laboratories in those countries must be performed strictly in containment level 3 facilities (CL3). However, the handling of pathogenic viruses must conform with any specific national recommendations: for example, in the case of HEV, the classification differs between countries and various international bodies. Indeed, the WHO and USA recommendations for this organism is biosafety level (BSL) 2, the Spanish recommendation is generally BSL 3 but not with all BSL 3 precautions as there is no evidence of aerosol contamination, and the British recommendation is BSL 3. This should be borne in mind when sending a sample likely to contain a virus to another laboratory. Only laboratories with the available CL3 facilities should handle any package suspected of containing a CL3 microorganism. Guidance should be sought from a national body, which provides advice on best practice procedures for the safe handling and containment of Hazard Group 2, 3 and 4 organisms. Note that many national guidelines are based on EU or international guidelines. If no national regulatory body of this type exists in a country, international or European guidelines, such as the WHO Laboratory Biosafety Manual 2nd Ed. (WHO, 2003), should be followed.

## Detection and identification of food and environmental virus hazards

Detection of viruses in food and environmental samples is challenging because of the large variety and complexity of samples, the possible heterogeneous distribution of a small number of viruses and the presence of components that may inhibit or interfere with virus detection (Goyal, 2006). A general flow chart for the analytical process (from sampling to final identification and characterization) for the detection of human enteric viruses is given in Fig. 2. It is necessary to separate and concentrate viruses from environmental materials before performing tests for detection (Sair *et al.*, 2002). As no standard procedure or systematic approach evaluating the adsorption of viruses onto different substrates has yet been developed, it is difficult to draw conclusions about the mechanisms involved in virus adsorption (Jin & Flury, 2002); consequently, establishing appropriate separation and concentration processes is even more demanding. Whatever the method used, the final concentrate should not be cytotoxic to cell cultures used in infectivity assays and

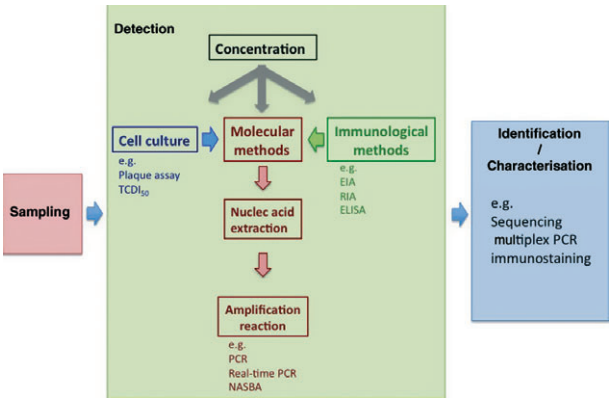


**Fig. 2.** Schematic diagram of the analytical process of detection and identification of environmental virus hazards. $TCDI_{50}$, median tissue culture infective dose assay; EIA, enzymatic immunoassay; RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; NASBA, nucleic acid sequence–based amplification.

should be free of any inhibitors, which may be co-extracted or co-concentrated from environmental samples (Goyal, 2006). A variety of biological and chemical substances that are present in environmental matter or are used during sample processing have been found to act as inhibitors, including polysaccharides, haeme, phenol and cations (Atmar, 2006). Known PCR inhibitors in shellfish extracts include glycogen and acidic polysaccharides (Schwab *et al.*, 1998).

For virological analysis of aerosols, the key issue is sample collection and preparation for the different detection procedures (mainly based on cell culture and/or molecular techniques). The sample size is generally 1–3 $m^3$ of air. Various approaches have been developed, based on the property of air-borne particles of attaching to every surface with which they enter into contact (Verreault *et al.*, 2008). There are three different principles underlying the most commonly used air samplers: membrane filtration, impact on solid surfaces followed by elution, or impingement in a liquid medium. The eluates produced can be further concentrated (Verreault *et al.*, 2008). Other methods for the virological analysis of aerosols include cyclone or electrostatic precipitators, and in recent years, the fear of bioterrorism has triggered assessments of various new methodologies (including mass spectrometry) able to identify dangerous species in the air. However, it is unlikely that such techniques will be suitable for routine environmental analysis in the near future, and furthermore, they require the establishment of very large databases of environmental samples.

To elucidate the fate of virus dispersed through air, surface monitoring should be also performed, because larger droplets tend to settle out. Surface sampling is most extensively used in health care settings and in food production to assess not only viral contamination but also the efficacy and correct application of disinfection procedures. For hard surfaces, a defined surface area (i.e. 10 or 36 $cm^2$) should be swabbed; the swab is then eluted, and the elute is processed as a liquid sample. Alternative methods are contact plates, which can be similarly eluted.

## Concentration of viruses

The aim of concentrating virus is to collect most of the virus present in the sample in a minimal volume (Cliver, 2008); this small sample can then be used for virus detection by molecular, immunological or cell culture–based methods (Fig. 2). Protocols for the concentration of viruses in water samples are generally based on four steps (Croci *et al.*, 2008): adsorption of viruses to a filter; elution of adsorbed viruses using a protein-rich buffer; reconcentration of viruses by flocculation, precipitation or filtration, and extraction of viruses, for example with chloroform. In solid samples (including foodstuffs), sample processing often starts with a washing step (in the case of fresh produce) or a homogenization step (in the case of, for example, shellfish); the virus is concentrated after this first step (Rodríguez-Lázaro *et al.*, 2007; Croci *et al.*, 2008). If appropriate, a minimal volume of a diluent can be added to favour dissociation of the virus from the solid matter but avoiding interference with subsequent virus concentration/extraction. For dispersion of the sample in the diluent, a suitable mixing technique is required. The following step is the removal of food solids from the extract by, for example, filtration or differential centrifugation. Concentration methods appropriate for a wide variety of matrices include adsorption elution, differential precipitation, ultracentrifugation and ultrafiltration (Rodríguez-Lázaro *et al.*, 2007).

## Detection methods used for human enteric viruses

Various approaches can be used to detect human enteric viruses in concentrated samples. They range from direct observation by electron microscopy to the detection of cytopathic effects in specific cell lines and of indirect diagnostic signals using immunological or molecular methods (Fig. 2).

Direct observation by electron microscopy is a laborious, painstaking and time-consuming task, is also subjective, and has a limited sensitivity (Atmar & Estes, 2001). The observation of cytopathic effects produced in specific cell lines is not always possible as some enteric viruses, notably NoV and HEV cannot be propagated in mammalian cell lines. Even when possible, this is not a simple or cost-effective technique. It may also require the adaptation of the virus before it can grow effectively (Pintó & Bosch, 2008). There are immunological tests such as enzymatic immunoassay, radioimmunoassay or enzyme-linked immunosorbent assay (ELISA), and many are commercially available for the main enteric viruses. However, their analytical sensitivity is still too poor for effective testing of environmental samples.

To overcome these various limitations and disadvantages, molecular techniques are now being used routinely in viral laboratories, and real-time quantitative PCR (q-PCR) has become the method of choice for the detection of enteric viruses. This approach has been reinforced by the recommendation of the international ISO/CEN committee CEN/TC275/WG6/TAG 4 that real-time PCR should serve as the basis for the forthcoming international standards for the detection of NoV and HAV (Lees and CEN WG6 TAG4, 2010). A large number of scientific studies using molecular methods for the detection of enteric viruses have already been published

(see Table S3 for a representative list of the published references).

q-PCR is a molecular technique that allows the quantification of the amount of the target template (i.e. specific virus) initially present in a sample (Heid *et al.*, 1996). Other major advantages of this technique include the closed-tube format that reduces the risk of carry-over contamination, the wide dynamic range of quantification and the possibilities for automation (Rodríguez-Lázaro *et al.*, 2007). However, q-PCR also suffers from some limitations. The volume used in the amplification reaction is very small; therefore, only concentration methods that can deliver a very small volume of the resulting nucleic acid solution (i.e. in the microlitre range) from a realistic food or environmental sample can be used. In addition, the quality of the nucleic acids is an important factor that directly affects the analytical sensitivity of the assay, and diverse compounds present in samples can inhibit the amplification reaction. The standardization of inhibition tests would help overcome this limitation once appropriate synthetic standards become available (La Rosa *et al.*, 2010). Finally, definitive international standardization efforts are required to guarantee effective implementation in the real-life analytical contexts.

Other detection options include the combination of cell culture or immunological methods and a molecular technique. The combination of a cell culture step and subsequent detection by a molecular technique such as RT-PCR or nucleic acid sequence–based amplification (NASBA) reduces the incubation periods and also allows the detection of viruses that grow without causing cytopathic effects (Table S3) (Dubois *et al.*, 2002; Duizer *et al.*, 2004b).

## Index viruses

Classic microbiological indicators such as faecal coliforms (*Escherichia coli* and enterococci) are the most commonly used indicators to evaluate both the level of faecal contamination and also efficiencies of the elimination of pathogens by water purification processes. However, the adequacy of these bacterial markers to indicate the presence and concentration of human viruses and protozoa cysts has been questioned in recent years (Lipp *et al.*, 2001; Tree *et al.*, 2003). EV, evaluated as cultivable enteric viruses, is the sole viral measure that has been included in past regulations. Results obtained by applying molecular techniques have shown that the presence of EVs does not significantly correlate with the presence of other pathogenic viruses that may be more abundant. Diverse groups of bacteriophages have also been suggested as indicators of viral contamination; this would allow in theory the use of simple assays for the detection of infectious viruses (Savichtcheva & Okabe, 2006; Love *et al.*, 2008), although their presence does not clearly correlate with the presence of specific viral pathogens (Formiga-Cruz *et al.*, 2003).

The improvement in molecular technologies for detecting viruses present in water and food has focused attention on new groups of DNA viruses that may be quantified with cost-effective molecular assays and are excreted in large quantities by the populations of widely divergent geographical areas. hAdV are often being detected in the environment (He & Jiang, 2005; Van Heerden *et al.*, 2005a; Katayama *et al.*, 2008; Muscillo *et al.*, 2008) and have been proposed along with human polyomaviruses as a molecular index of viral contamination of human origin (Puig *et al.*, 1994; Pina *et al.*, 1998; Bofill-Mas *et al.*, 2000). Testing for hAdV is of interest for two different reasons: both to assess the presence of this human pathogen itself and also as a more general indicator. Most of the population is seropositive for the most common AdV and also for the human polyomaviruses JCPyV and BKPyV. The presence of these viruses in water therefore presents only a low risk for healthy immunocompetent populations (Bofill-Mas *et al.*, 2001). Specific animal AdV or polyomaviruses have been also proposed as microbial source tracking tools (Hundesa *et al.*, 2006, 2009).

hAdV and JCPyV have been found in 98% of the sewage samples analysed from widely diverse geographical areas around the world (Bofill-Mas *et al.*, 2000), with concentrations of about $10^5$–$10^7$ genome equivalents (GE) $L^{-1}$. The concentrations are generally higher for hAdV than for JCPyV. These viruses have also been commonly found in river water and have been used as a marker for the evaluation of the efficiency with which water treatment plants eliminate virus (Bofill-Mas *et al.*, 2006; Albinana-Gimenez *et al.*, 2009a).

q-PCR methods have been developed for the detection of hAdV in sewage, shellfish, river water and drinking water (Puig *et al.*, 1994; Pina *et al.*, 1998; Formiga-Cruz *et al.*, 2002; Albinana-Gimenez *et al.*, 2009b) and in sea water (Calgua *et al.*, 2008). hAdV has also shown to be very stable in the environment and resistant to water treatments (Thompson *et al.*, 2003; Mena & Gerba, 2009). A very high proportion of environmental or shellfish samples presenting human viral pathogens contain AdV (Formiga-Cruz *et al.*, 2002); they are the most abundant viruses, as assessed by PCR, and are regularly found in faecal contamination. In a study using q-PCR, hAdV was detected in 100% of the urban sewage samples analysed at concentrations of $10^4$–$10^5$ GE $mL^{-1}$, and these viruses were still present in treated effluents at concentrations of $10^2$–$10^3$ GE $L^{-1}$. The biosolids generated accumulated $10^2$–$10^5$ AdV GE $g^{-1}$. JCPyV also were

quantified, and the concentrations found were $10^3$–$10^4$ GE $mL^{-1}$ in urban sewage, $10^2$–$10^3$ GE $L^{-1}$ in treated effluent and $10^3$ GE $g^{-1}$ in the biosolids generated (Bofill-Mas *et al.*, 2006).

The application of index viruses in future regulations on the microbiological quality of water should be a step forward for improving the control of the environment, food and water. However, this would require further studies, including epidemiological studies, for the definition of acceptable values of index viruses and to identify where such values would be appropriate.

## Evaluation and interpretation of test results

One of the major differences between the study of the presence and enumeration of bacteria and that of viruses in food and in the environment is the availability of a "gold standard" method for detection. Classical culture-based techniques are considered the gold standard for the detection of bacteria, but the situation is exactly the opposite for the detection of viruses, since no accepted standard method exists. The lack of a defined and consensus standard method for the detection and quantification of viruses is hindering and slowing the adaptation of quantitative viral risk assessment (QVRA) models for food and food environments. Therefore, the establishment and application of a common and validated method for virus detection would make a large contribution to the effective harmonization of QVRA studies. The combination of cell culture and PCR generally produces higher viral counts than those resulting from cell culture methods (i.e. plaque-forming units or $TCID_{50}$) and could be considered a *de facto* standard (Havelaar & Rutjes, 2008).

### Validity of molecular detection methods

The reliability of the results produced by molecular techniques is undermined by the lack of standard methods for the detection of viruses in environmental samples and the wide diversity of viruses, matrices, assays and recovery efficiencies described. Molecular techniques, if used with the appropriate quality controls, could allow substantial progress in the control of viral contamination of environment and food. These quality controls must include at least one negative and one positive reaction control, one negative and one positive process control and an internal or external amplification control (Hoorfar *et al.*, 2004; Costafreda *et al.*, 2006; Rodríguez-Lázaro *et al.*, 2007; Pintó & Bosch, 2008; D'Agostino *et al.*, 2011; Diez-Valcarce *et al.*, 2011a, b; Martínez-Martínez *et al.*, 2011) (Table 1). Controls for the estimation of the efficiency of the concentration and/or extraction procedures are also very important. Several approaches have suggested the use of nonpathogenic virus surrogates, with similar structural characteristics and which are not present naturally in the samples to be tested. As examples, Mengo virus $MC_0$ (Costafreda *et al.*, 2006) and feline calicivirus and murine NoV-1 (Cannon *et al.*, 2006) have been proposed as appropriate surrogates for HAV and human NoV, respectively.

**Table 1.** Analytical controls for (RT) real-time PCR-based detection of viral hazards in food matrices

| |
|---|
| *Process controls* |
| Processing Positive Control (PPC): A negative sample spiked with sufficient viral target and processed throughout the entire protocol. A positive signal should be obtained indicating that the entire process was correctly performed |
| Processing Negative Control (PNC): A negative sample spiked with sufficient amount of nontarget or water and processed throughout the entire protocol. A negative signal should be obtained, indicating the lack of contamination throughout the entire process. For example, the inclusion of encapsidated RNA (or DNA) or bacteriophages |
| Environmental Control: A tube containing the master mixture or water left open in the PCR set-up room to detect possible contaminating nucleic acids in the environment |
| *Amplification controls* |
| Positive PCR control: A viral template known to contain the target sequence. Positive amplification indicates that amplification was performed correctly. It could be used a natural virus or chimerical nucleic acids |
| Negative PCR control (or No Template Control -NTC- or Reagent Control or Blank): Including all reagents used in the amplification except the template nucleic acids. Usually, water is added instead of the template. A negative signal indicates the absence of specific contamination in the amplification assay |
| External Amplification control (EAC): An aliquot of a solution of control DNA, containing a defined quantity or copy number, added to an aliquot of the nucleic acid of the extracted sample and analysed in a separate reaction tube. A positive signal indicates that the sample nucleic acid extract did not contain any inhibitory substances |
| Internal Amplification Control (IAC): Chimerical nontarget nucleic acid added to the master mix to be co-amplified with the same primer set as the viral target but with an amplicon size visually distinguishable or different internal sequence region from the target amplicon. The amplification of the IAC both in the presence and in the absence of the target indicates that the amplification conditions are adequate |

Adapted from Rodríguez-Lázaro *et al.* (2007), Pintó & Bosch (2008), Bosch *et al.* (2011) and D'Agostino *et al.* (2011).

Negative results obtained using correctly designed and controlled PCR assays can provide robust evidence for the absence of pathogens or indicators in the samples analysed with strong implications for risk assessment. Such negative results from well standardized and highly sensitive PCR assays may be acceptable and may facilitate the implementation of potential regulations requiring the absence of pathogens from defined sample volumes, as is the case for food or water safety criteria. More studies are needed to evaluate the significance of positive results, because the differing sensitivities of diverse techniques, like infectivity assays if available, do not allow a definitive evaluation of the infectious capability of the viral genomes detected. Also, if viral measures are considered for regulations concerning the microbiological quality of bathing water or other environmental samples, epidemiological studies would be needed to establish acceptable limits for index viruses.

## Infectious particles vs. PCR GE: implications for public health

Viral infectivity is defined as the capacity of viruses to enter the host cell and exploit its resources to replicate and produce progeny infectious viral particles (Black, 1996; Rodríguez *et al.*, 2009), which may lead to infection and subsequent disease in the human host. Therefore, the information required in risk assessment studies is the number of viral particles with infective capacity. Obviously, cell culture–based methods are the soundest methodologies for the estimation of the number of infective particles. However, as indicated earlier, there are no available culture models for some of the most significant food and environmental virus hazards, notably human NoV, HEV and even wild-type HAV. In these cases, only molecular methods are available, but although RTq-PCR is a quantitative and sensitive tool, it cannot distinguish between infective and noninfective viruses (Richards, 1999). This limits its usefulness for public health purposes. The ratio between GE and infectious particles has been reported to increase with the time, is strongly dependent upon water and climatic conditions and virus type, and can vary from 70 : 1 to 50 000 : 1 for EV in natural surface water (Rutjes *et al.*, 2005) and in artificial ground and surface waters (de Roda Husman *et al.*, 2009). For example, wastewater can contain up to 1500 GE HAstV $L^{-1}$ but do not show any infective capacity. To overcome this limitation, several different approaches based on (RT) PCR have been assessed (reviewed in Rodríguez *et al.*, 2009; see Table 2 for examples). However, it is unclear whether any direct PCR method can satisfactorily assess viral infectivity.

## Risk assessment

As stated earlier, QVRA is theoretically a powerful statistical tool for the estimation of the probability of a viral infection or disease based on exposure of the human host to the viral hazard and for establishing the dose–response relationship (Haas, 1983; Haas *et al.*, 1993). Consequently, QVRA has been used for exposure to various virus hazards in different environmental matrices, mostly for aquatic environments (e.g. Van Heerden *et al.*, 2005b).

In general, the risk analysis framework (FAO and WHO, 2006) consists of hazard identification, exposure assessment, hazard characterization and risk characterization, which should identify and preferably quantify the risk. In the case of QVRA for environmental exposure, this framework reads as follows: (1) hazard identification: the identification of viral agents that may be present in a particular environmental matrix and are capable of causing adverse health effects; (2) exposure assessment: quantitative evaluation of the likely intake of viral agents via exposure to environmental sources; (3) hazard characterization: quantitative evaluation of the nature of the adverse effects associated with the viral agents that may be present in the environment one is exposed to and; (4) risk characterization: the integration of hazard identification, exposure assessment and hazard characterization into a risk estimate of the likelihood and the severity of the adverse effects in a given population with attendant uncertainties.

Various viral characteristics, as described in this paper, are important determinants of the risk of infection or disease upon exposure: numbers (or dose), infectivity and pathogenicity to humans. Application of QVRA has been rendered difficult by the lack of culturing systems and low environmental levels of viruses that present a possible public health risk but cannot be typed or quantified. Moreover, standardized methods for quantification of virus hazards in different environmental matrixes and dose–response models for the main environmental virus hazards are not available. For reliable quantification of viruses in food and environmental matter, various factors need to be determined: the detection efficiency of the assay used, the controls appropriate for accurately measuring both the true concentration and the release of virus into the environment, and the contamination of the food (Pintó & Bosch, 2008; Pintó *et al.*, 2009). This is of the utmost importance for unculturable viruses, such as HEV and human NoV, for which only molecular quantitative detection methods are available. The raw numbers of GE, which are the data generated by such methods, must be corrected for the efficiency of the concentration and nucleic acid extraction steps and the capacity of the

**Table 2.** Molecular-based methods used for assessing viral infectivity

| Method | Treatment | Detection | Type of sample | Target virus | References |
|---|---|---|---|---|---|
| Molecular | Proteinase and RNase | RT-PCR | Cell culture | FCV HAV, MNoV, poliovirus 1, | Nuanualsuwan & Cliver (2002, 2003); Baert *et al.* (2008) |
| | Proteinase and RNase | qNASBA | Stool samples and cell culture | NoV, FCV | Lamhoujeb *et al.* (2008, 2009) |
| | RNase protection assay | qRT-PCR | Stool samples and cell culture | NoV, FCV | Topping *et al.* (2009) |
| | | 5′ NTR RT-PCR | Cell culture | HAV | Bhattacharya *et al.* (2004); Li *et al.* (2002, 2004) |
| | | Long target region (LTR) qRT-PCR | Cell culture | HAV, poliovirus 1, F-specific RNA phages | Li *et al.* (2002); Simonet & Gantzer (2006a, b) |
| Cell culture + molecular | Attachment to cell monolayer | RT-PCR | Cell culture | HAV, poliovirus 1, FCV | Nuanualsuwan & Cliver (2003) |
| | Virus replication in cell culture (ICC: integrated cell culture) | RT-PCR | Different types of water, sewage effluent, faecal specimens and cell culture | AdV, AstV, EV, poliovirus, RV, HAV, MS2 | Blackmer *et al.* (2000); Chapron *et al.* (2000); Jiang *et al.* (2004); Ko *et al.* (2003, 2005); Lee & Kim (2002); Lee & Jeong (2004); Li *et al.* (2009); Nuanualsuwan & Cliver (2003); Reynolds *et al.* (1996); Shieh *et al.* (2008) |
| Immunological +molecular | Antibody capture | RT-PCR | Different types of water, faecal samples and cell culture | HAV, NoV, poliovirus 1, FCV | Gilpatrick *et al.* (2000); Myrmel *et al.* (2000); Schwab *et al.* (1996) |
| | Immunomagnetic separation | qRT-PCR | Artificially contaminated groundwater | HAV | Abd El Galil *et al.* (2004) |

RT-PCR, reverse transcriptase PCR; qRT-PCR, reverse transcriptase real-time PCR; qNASBA, real-time nucleic acid sequence–based amplification; FCV, feline calicivirus; mNoV, murine NoV.

enzyme involved in the amplification-based detection. A formula for the estimation of exposure to viruses in food matrices has been proposed by Havelaar & Rutjes (2008).

Following exposure assessment, hazard characterization is possible using dose–response models, which describe the relationship between virus particles detected and the probability of disease. Viral dose–response models are based on three basic biological assumptions: single hit, independent action and random distribution (FAO and WHO, 2006). Using these assumptions, three different models can be applied to environmental virus hazards (Haas, 1983; Teunis & Havelaar, 2000; Zwietering & Havelaar, 2006). For example, Pintó *et al.* (2009) estimated the relationship between HAV numbers in frozen coquina shellfish involved in two hepatitis outbreaks and the risk for human health. However, for HAV, immunity needs to be taken into account. Similarly, for human NoV that only induces short-lived immunity, risk assessment should also take into account the observation that a proportion of the population is resistant to infection with NoV genogroup GI (Hutson *et al.*, 2002; Lindesmith *et al.*, 2003; Rockx *et al.*, 2005) or GII (Thorven *et al.*, 2005; Larsson *et al.*, 2006).

The viral risk can thus be estimated from the information obtained from an exposure assessment and the dose–response relationship (Zwietering & Havelaar, 2006). In addition, the estimation of the disease incidence can be also extrapolated to estimates of disease burden and costs (Havelaar & Rutjes, 2008). Published risk assessments for environmental viruses mainly concern water-borne or food-borne exposure, but other routes may be considered as well. For food-borne viruses, the EU research project '*Integrated monitoring and control of food-borne viruses in European food supply chains*' (KBBE 213178; VITAL; www.eurovital.org) has been launched to develop proactive integrated monitoring and risk management strategies for the control of viral contamination of food supply chains. Moreover, a network of food and environmental virologists, under COST Action 929, ENVIRONET (www.cost929-environet.org), has been established to improve our knowledge and the role of the environment and food in the transmission of enteric viral disease.

## Concluding remarks and recommendations

Environmental virus hazards are increasingly recognized as a cause of illness in all age groups. Caliciviruses (NoV), AdV, EV, RV, HAV and HEV are the most common causes of illness because of environmental exposure. The major routes of exposure to environmental viruses involve human or animal faeces, surface water or sewage, especially irrigation waters in relation to crops, and fresh and noncooked produce along the food chain, and in particular bivalve molluscs, which filter feed in virus-contaminated waters. In addition to the risks associated with the contamination of environmental or food matrixes with viruses of human origin, there are also pathogenic viruses that are zoonotic, i.e. of animal origin and transmitted from animals.

Education of populations at risk should give particular attention to describing potential virus contamination routes, especially for those working with water, sewage, faeces and food. Education about risks is also important for health care workers and consumers. The most important preventive measures include the improvement of hygienic conditions during harvesting, processing and handling of potentially contaminated environmental matter. Legislation on handling and treatment of water, sewage and foods should be adapted as needed to reduce the risk of environmental virus contamination. The systems for sewage treatment and the codes of practice for agricultural use of sewage and surface water should be reviewed to address these issues.

Methods related to virus purification and detection of viral particles should be improved such that survival of human pathogenic viruses in the environment can be followed reliably. In parallel, techniques should be further developed for effective virus inactivation and decontamination of environmental materials suspected to pose a risk. When human disease is caused by environmental exposure to viruses, and also for the assessment of virus contamination in environmental matter, virus monitoring is required, and it may be beneficial to implement a virus surveillance strategy. Unfortunately, this is not straightforward. Samples must represent the environmental matter being studied, and tests for specific virus hazards may need specific sampling and sample processing techniques. Safe and efficient transport and laboratory practices are of utmost importance for laboratory workers and the outcomes of prevention and control measures.

The development of a suitable detection technique for a virus in an environmental sample requires a targeted specific approach. This generally starts with the separation and concentration of the virus. Appropriate concentration methods include adsorption elution, differential precipitation, ultracentrifugation and ultrafiltration. Then, various virus identification methods can be used; possible methods range from classical techniques like cell culture and electron microscopy to molecular techniques like RT-PCR and microarrays, and combinations may also be used. Development of a general method that can be applied to different matrices is difficult and, indeed, may not be feasible. Nevertheless, the CEN/TC 275- Food Analysis, Horizontal Methods; Working Group 6, Technical Advisory Group 4 (CENTAG4) is pursuing efforts for the develop-

ment of such horizontal methods for detection of viruses in foods.

To evaluate the extent of environmental virus contaminations, it can be helpful to test for particular index viruses, whose presence correlates with the presence of other pathogenic, viruses that may be more abundant. Because of their wide applicability and high level of sensitivity and specificity, molecular techniques are most commonly used for the detection of environmental virology. Powerful molecular techniques can be extremely valuable if appropriate controls are used. However, for estimation of the true virus hazard, the detection of GE, which is the output of molecular techniques, has to be related to the quantity of infectious particles present.

To estimate the probability of a viral infection, the statistical tool QVRA can be used. This involves virus hazard identification, exposure assessment, hazard characterization and risk characterization. Satisfactory exposure assessment requires a reliable quantification of the virus present in the environmental material. For reliable quantification of virus in environment, the detection efficiency of the assay used must be determined, and appropriate controls must be employed to determine accurately the true concentration and release of virus in the environment. In conclusion, the study of environmental virus hazards is extremely important to estimate the public health risks associated with viruses.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Sampling methods used for detection viral hazards in food matrices.

**Table S2.** Sampling methods used for detection viral hazards in water samples.

**Table S3.** Detection methods for viral hazards in different environmental matrices.

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# Direct potable reuse: a future imperative

Harold L. Leverenz, George Tchobanoglous and Takashi Asano

## ABSTRACT

As a result of population growth, urbanization, and climate change, public water supplies are becoming stressed, and the chances of tapping new water supplies for metropolitan areas are getting more difficult, if not impossible. As a consequence, existing water supplies must go further. One way to achieve this objective is by increased water reuse, particularly in supplementing municipal water supplies. Although water reuse offers many opportunities it also involves a number of problems. A significant cost for nonpotable water reuse in urban areas is associated with the need to provide separate piping and storage systems for reclaimed water. In most situations, the cost of a dual distribution system has been prohibitive and thus, has limited implementation for water reuse programs. The solution to the problem of distribution is to implement direct potable reuse (DPR) of purified water in the existing water distribution system. The purpose of this paper is to consider (a) a future in which DPR will be the norm and (b) the steps that will need to be taken to make this a reality. Following an overview, the rationale for DPR, some examples of DPR projects, technological and implementation issues, and future expectations are examined.

**Key words** | direct potable reuse, engineered storage buffer, potable reuse, water reuse

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## DIRECT POTABLE REUSE: AN OVERVIEW

Direct potable reuse (DPR) refers to the introduction of purified water, derived from municipal wastewater after extensive treatment and monitoring to assure that strict water quality requirements are met at all times, directly into a municipal water supply system. The resultant purified water could be blended with source water for further water treatment or even direct pipe-to-pipe blending of purified water and potable water. DPR offers the opportunity to significantly reduce the distance that purified water would need to be pumped and significantly reduce the head against which it must be pumped, thereby reducing costs. The other significant advantage of DPR is that it has the potential to allow for full reuse of available purified water in metropolitan areas, using the existing water distribution infrastructure.

A general flow diagram for alternative potable reuse strategies is shown on Figure 1. As shown, two DPR options are available. In the first option (heavy solid black line), purified water is first placed in an engineered storage buffer (ESB). From the ESB, purified water can either be blended with the water supply source prior to water treatment or can be blended directly with treated potable water. In the second option (heavy dashed back line) purified water, without the use of an ESB, can be blended in either of the two locations discussed for option 1. As will be discussed later, implementation of option 2 would entail more extensive reliability measures and effective on-line continuous monitoring. The concept and role of the ESB is considered in the following discussion.

### Engineered storage buffers for quality assurance

An important element of a DPR system is the ability to provide water of a specified quality reliably all the time. Because of the past limitations in providing this level of quality control in real-time and the large number of unknown factors, there was a preference for indirect potable reuse (IPR) projects instead of DPR projects. IPR systems make use of an environmental buffer, such as a surface reservoir or groundwater basin, to store water and ostensibly provide enhanced

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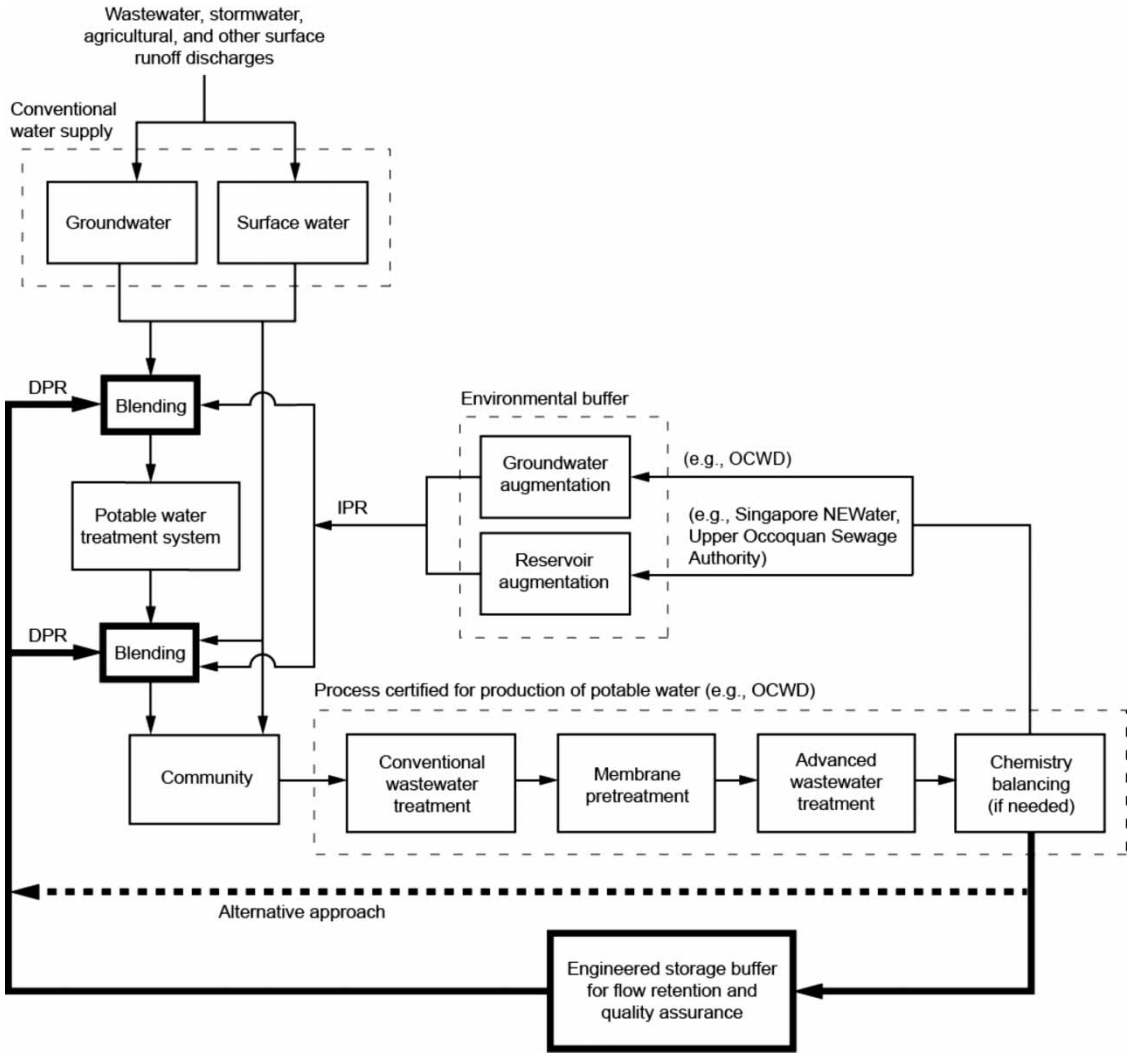


**Figure 1** | Flow diagram for alternative direct potable reuse schemes (Tchobanoglous *et al*. 2011).

quality. In early IPR projects where the product water was not of the highest quality, the environmental buffer was thought to have provided a level of *in situ* advanced treatment. Further, the environmental buffer was presumed to provide loss of water identity and a measure of safety, in that it provided time to correct issues in the event that off-spec product water was detected.

However, when water is treated to a high level of purity, placement into an environmental system may not result in improved water quality, and can instead expose the purified water to potential environmental contaminants. Thus, when purified water can be produced using a system with proven performance and reliability and the quality can be validated rapidly with extensive monitoring systems, a relatively small ESB, if any, may be sufficient for use prior to blending into the potable water system.

An additional implication of the ESB concept is that, with some additional infrastructure, an existing IPR system could blend the purified product water directly with the area's general water supply system, allowing for greater flexibility in system operation. For example, when there are periods of purified water production in excess of the immediate potable demand, purified water could be placed into long-term environmental storage, such as aquifer recharge. Additional discussion on ESBs is presented in the 'Technical issues' section of this paper.

### Water is water

Understandably, DPR may be the most difficult category of water reuse applications for the community to accept. One of the dilemmas in considering DPR has been the perception, even among water professionals, that nearly any water obtained from the environment, i.e., natural, is pure and better (Lohman 1988). However, the distinction that natural water is pure and better is no longer valid in many areas, mostly due to intentional and unintentional discharges of wastewater and agricultural and urban runoff. As a result, much of the research that originally addressed potable reuse has become of equal relevance to drinking water supplies taken from most water bodies. Thus, the sage words of Dr Lucas van Vuuren have successfully withstood the test of time over 40 years: 'Water should not be judged by its history, but by its quality' (Haarhoff & van der Merwe 1996).

### A future imperative

It is inevitable that purified water will be used as a source of potable water supply in the future. Implementation of DPR will require a confidence in, and reliance on, the applied technology to always produce water that is safe and acceptable to consume. Designing interconnected water supply, collection, treatment, purification, and distribution systems has the benefit of providing maximum flexibility in the event of expected or unexpected shortages of natural water supply. Once a decision has been made to augment an existing water supply with purified water, the technical and implementation issues introduced in this paper must be considered. Further, the concepts described in this paper can also be applied in developing countries when provisions are made for reliable power supply and operation and maintenance for their vital water supplies.

## RATIONALE FOR DIRECT POTABLE REUSE

In the past, it has been standard practice that whenever additional sources of water supply are necessary but not readily available, nonpotable water reuse options have been explored using recycled water. For example, nonpotable water reuse applications, such as agricultural and landscape irrigation, are major options for planned reuse. As a result of the preference for nonpotable reuse, water reuse applications in the United States, in order of descending water volume, are: (1) agricultural irrigation; (2) industrial recycling and reuse; (3) landscape irrigation; (4) groundwater recharge; (5) recreational and environmental uses; (6) nonpotable urban uses; and finally, (7) potable reuse (Asano 1991; Asano *et al*. 2007). However, most of the economically viable nonpotable reuse opportunities have been exploited. For example, the typical cost for parallel distribution of tertiary-treated recycled water is 0.3 to \$1.7/$m^3$ whereas the typical cost for purified water, which could be added directly to the distribution system, is 0.6 to \$1.0/$m^3$ (Tchobanoglous *et al*. 2011).

### Indirect planned and unplanned potable reuse

*Planned* IPR includes groundwater recharge operations, such as Orange County Water District in California and the Occoquan Reservoir in northern Virginia (Asano *et al*. 2007). Planned IPR will continue to be of great importance in supplementing water supplies in the United States and elsewhere in the world. *Unplanned* IPR, in the cities and towns along the Colorado River as an example, occurs when treated wastewater is discharged to surface and groundwater that is subsequently used for municipal water supply. Thus, much of the research that originally addressed potable reuse is becoming of equal relevance to drinking water supplies taken from water bodies used for discharge of wastewater and runoff.

### Factors limiting nonpotable and indirect potable water reuse

While there has been a clear preference for nonpotable and IPR applications, a number of factors are making it less feasible to further increase water reuse in these applications. Important limiting factors for agricultural and landscape irrigation, and IPR are listed in Table 1. Although agricultural irrigation is currently the largest user of recycled water, it is expected that this will change with the world-wide trend towards urbanization, especially near coastal areas. For example, the City of Los Angeles currently discharges about 1.5 $Mm^3$/d (400 Mgal/d) of treated wastewater to the Pacific

Ocean. Further, the energy to provide water supply to some areas is excessive compared to the energy to purify water. For example, the energy required to provide 1,234 $m^3$ (1 ac-ft) to an Orange County water system is: ocean desalination = 3,700 kWh (kilowatt-hour); State Project water = 3,500 kWh; Colorado River water = 2,500 kWh; purified water = 800 to 1,500 kWh (Tchobanoglous *et al.* 2011).

### Factors favoring direct potable reuse

In addition to the limiting factors identified in Table 1, there are a number of factors that support the implementation of DPR in the future. For example, drought events are expected to become more extreme due to climate change and the potential use of purified water for potable supply offers improved overall water supply reliability in coastal metropolitan areas. Another consideration is that as the reality of unplanned IPR and concern about the quality of existing water supplies becomes more transparent and understandable to the public, there will be increased pressure to provide water of the highest quality for public consumption. Advances in treatment technology over the last decade have made it possible to produce high quality purified water with advanced water treatment processes. Additional considerations that support DPR are summarized in Table 2. Given the factors presented in Tables 1 and 2, it is clear that there is a need in some regions to consider alternatives to conventional water supply and nonpotable water reuse applications.

## REVIEW OF DPR SYSTEMS

Some DPR systems that are currently in operation and/or under construction are highlighted in this section. These example projects are important because 'the treatment process flow diagrams and treatment technologies employed have been accepted by various regulatory authorities as being able to produce safe potable drinking water, and … the implementation of these projects has been accepted by the public' (Tchobanoglous *et al.* 2011). Therefore, the focus of this section is primarily on treatment technologies and not the removal of specific constituents.

### Typical flow diagrams for DPR

Representative treatment process flow diagrams from (1) Windhoek, Namibia; (2) Big Springs, Texas; (3) Cloudcroft, New Mexico; and (4) Orange County Water District (OCWD) Groundwater Replenishment System (GWRS), Fountain Valley, California for potable reuse are presented on Figure 2. The Windhoek, Namibia DPR facility, shown

**Table 1** | Factors that have limited nonpotable and indirect potable reuse

| |
|---|
| Agricultural irrigation |
| • The long distance between the municipal recycled water supplies and the major agricultural demand areas.<br>• The cost and disruption to construct pipe systems to convey recycled water.<br>• The need to provide winter recycled water storage facilities further limits agricultural reuse.<br>• Historically, the value of water from surface and groundwater supply sources has not reflected the true costs of providing the supply, resulting in a distinct economic disadvantage for the production of recycled water. |
| Urban landscape irrigation |
| • Landscape irrigation may not be economically feasible due to the dispersed nature of the demand.<br>• The cost of providing parallel distribution of recycled water supply is high due to the fact that the distance between large users in most communities is great. Further, most of the water is consumed by small users that cannot be served efficiently and or economically. |
| Indirect potable reuse (IPR) projects |
| • Communities that lack suitable hydrogeology for groundwater recharge may not be able to implement IPR projects.<br>• For surface water augmentation, blending and residence time requirements may limit IPR applications to large reservoirs (which are not available to many communities). |

**Table 2** | Factors that favor direct potable reuse

- Need for a separate recycled water distribution system is avoided.
- Alternative sources of water supply are often either of poor quality or prohibitively expensive.
- Traditional sources of surface water and groundwater supply are being limited.
- With advanced treatment technology it is now possible to remove contaminants effectively and reliably to extremely low levels that have no known health concerns.
- Purified water is a reliable source of supply which exists in close proximity to the demand.
- Communities that lack suitable hydrogeology for groundwater recharge cannot implement IPR projects.
- DPR with purified water is potentially less costly than the use of tertiary-treated recycled water for irrigation.
- DPR may require less energy than is required for other water supply sources.
- DPR avoids potential water quality issues associated with groundwater and surface water sources.
- Current technology is sufficient to replace the environmental buffer with an engineered storage buffer through a combination of monitoring, storage, and treatment reliability measures.

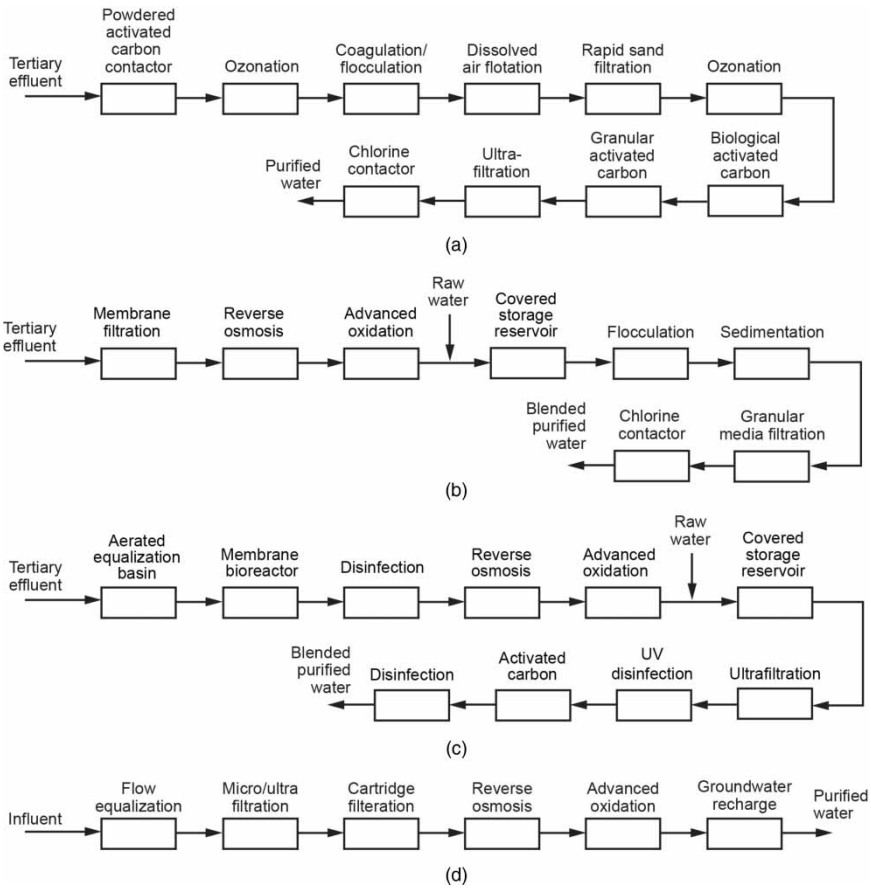


**Figure 2** | Representative treatment process flow diagrams for potable reuse: (a) Windhoek, Namibia; (b) Big Springs, Texas; (c) Cloudcroft, New Mexico; and (d) Orange County Water District (OCWD) Groundwater Replenishment System (GWRS), Fountain Valley, California.

on Figure 2(a), has been in operation since 1997 and replaced the previous treatment facility, which had been in operation since 1968. It should be noted that all of the flow diagrams in Figure 2, with the exception of Figure 2(d), are consistent with the generalized conceptual DPR flow diagram given on Figure 1. Although the purified water from the GWRS

system, shown on Figure 2(d), is used for groundwater recharge, the treatment process flow diagram is included as a benchmark for water quality, as the water has been determined to be safe for direct potable reuse (Burris 2010).

### Assessment of flow diagrams for DPR

In reviewing the flow diagrams presented in Figure 2, it is interesting to note that a number of different unit processes have been employed for the removal of the constituents of concern in wastewater. For the near future, it is anticipated that the treatment processes employed in these flow diagrams will serve as a benchmark for the development of alternative process flow diagrams for DPR. As new treatment process flow diagrams are developed it will be important to assess the need for and size of the ESB, based on system reliability and the use of appropriate monitoring equipment and analytical techniques.

## TECHNICAL ISSUES IN DPR

The technology required for advanced wastewater treatment, capable of producing an effluent of sufficient quality that is suitable for potable reuse, has been a reality for more than 40 years. However, over the last decade, the ability to produce purified water reliably from tertiary and advanced effluent at the municipal scale has become technically and economically feasible. As more communities and water agencies begin to explore the feasibility of DPR, some of the technical issues that must be addressed include appropriate treatment process configurations, features of ESBs, process reliability, and monitoring requirements. These topics are considered below along with some research needs.

### Treatment process configurations for purified water production

The combination of improved technology and analytical capabilities has made it possible to validate the concept that water can be purified using several alternative process flow schemes. The basic system used to purify water consists of several processes collectively referred to as advanced treatment. The current advanced treatment scheme has evolved over time, and now commonly includes microfiltration, reverse osmosis, and advanced oxidation, as shown on the flow diagrams presented in Figure 2. Major innovations in the future are expected to include improvements in overall process cost and efficiency, such as demineralization processes that minimize brine formation and operate with reduced energy input.

### Features of ESBs

ESB designs can be stand-alone facilities or incorporated into the transport and distribution system, depending on site-specific factors and needs. Stand-alone storage buffers may take a variety of forms varying from well-defined engineering structures to natural or constructed confined groundwater aquifers. The specific design of the ESB will be a function of several factors, including: (1) site-specific constraints; (2) capabilities of the monitoring and constituent detection system; (3) flow rate and degree of flow equalization required; and (4) safety factors. Important features of the ESB include:

- fully controlled environment,
- contained to prevent contamination and evaporative losses,
- no source of contaminants from within the buffer itself,
- ability to divert flow out of the buffer as needed,
- accommodation of monitoring and sampling equipment,
- well-characterized and optimized hydraulics, and
- high level of security.

In general, the storage requirements will be controlled by the time required for constituent analysis and overall reliability of the monitoring system. Purified water must be retained in the ESB for sufficient time to validate the quality of the water for specified constituents and surrogate measures prior to blending into a potable water supply for consumption.

### Measures to enhance reliability

The pretreatment processes used for production of the feed water to advanced treatment and purification processes

must be refined to achieve the highest level of reliability possible. Optimizations of existing processes as well as incorporation of new facilities, such as full flow equalization, are needed to produce a consistent and stable input. Measures that can be taken to enhance the reliability of a DPR system include:

- enhanced source control,
- enhanced fine screening,
- elimination of untreated return flows,
- flow equalization,
- operational mode for biological treatment,
- improved performance monitoring,
- ongoing pilot testing and
- reformulation of consumer products for improved biodegradability.

The discharge of substances known to be difficult to treat can be reduced or eliminated with enhanced source control programs. Enhanced fine screening improves the performance of biological treatment processes. The elimination of return flows is significant with respect to achieving effective nitrogen removal. Flow equalization, coupled with operational mode of the biological treatment process, is effective in the treatment of trace organics. Improved process monitoring will enhance overall process performance. Pilot testing is used to keep abreast of the latest technological developments. Elimination of consumer products that end up in wastewater that are not amenable to treatment is the long-term goal.

### Monitoring and constituent detection

While there have been a number of recent improvements in online monitoring and constituent detection, it is not, at present, feasible to provide real-time monitoring of all constituents of concern. However, the identification of surrogate and indicator constituents that can be used to assess performance reliability of key unit processes can be used in place of direct measurements for all constituents of interest. The use of indicators and surrogates is somewhat site specific and will need to be established for individual treatment operations (Drewes *et al.* 2010). However, after these parameters are established they can be used to enhance the monitoring program through rapid detection programs. The ability to detect constituents of concern rapidly will reduce the overall size of the ESB facilities that are used for quality assurance.

Monitoring at specific locations is used: (1) to assess process performance and reliability; (2) for process control; and (3) to verify compliance with public health or other regulatory requirements. As described previously, the ESB is a key monitoring location because it may be the final safeguard prior to distribution in the potable water system. Thus, the development of the monitoring program needs to be planned carefully to ensure that all constituents of importance can be assessed in the product water with sufficient speed and accuracy to justify the size and design of the ESB facilities. It is at this point that off-spec water would be diverted to an alternate location, such as the wastewater treatment facility or a specified point in the purification process.

### Research needs

Although the technical feasibility of DPR is well established and will only improve in the future, areas of technical research that will enhance and hasten the adoption of DPR include (1) development of sizing criteria for ESBs; (2) treatment train reliability; (3) blending requirements; (4) enhanced monitoring techniques and methods; and (5) effectiveness of equivalent advanced treatment trains. Research on public acceptance will also be an important adjunct to and will be complementary to the technical areas of research discussed in this paper.

## FUTURE TECHNICAL DEVELOPMENTS

Future technical developments that will impact DPR include the need for enhanced wastewater treatment, the development of alternative treatment processes, and integrated wastewater treatment plant design for DPR.

### Enhanced wastewater treatment

It is important to consider that all water discharged to the surface and groundwater, from point and non-point sources, is basically a form of IPR. In recent surveys of

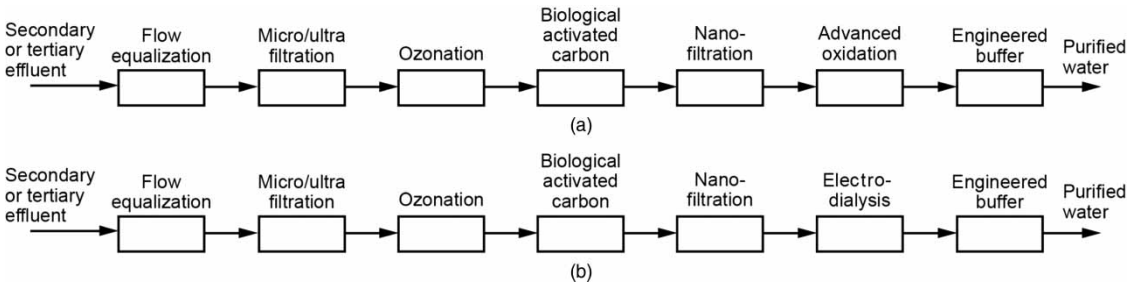


**Figure 3** | Alternative advanced treatment flow diagrams with trace organic removal by (a) ozonation, biological activated carbon, nanofiltration, and advanced oxidation and (b) ozonation, biological activated carbon, nanofiltration, and electrodialysis.

surface and groundwater quality by the US Geological Survey (Kolpin *et al*. 2002; Barnes *et al*. 2008), it was concluded that essentially all surface and groundwater are contaminated with chemicals commonly associated with wastewater, such as pharmaceuticals. In the future, it is anticipated that surface and groundwater discharges will need to comply with much more stringent discharge requirements to protect sensitive environmental species and ecosystems. The level of treatment needed to protect environmental species and ecosystems may, in some cases, be higher than that needed for DPR. Thus, the implementation of DPR may make more sense environmentally than the discharge of purified water to the aquatic environment.

## Alternative treatment processes for direct potable reuse

One of the major problems with most common DPR treatment schemes employing reverse osmosis is the management of brine, especially in inland locations. To deal with this issue, a variety of new advanced treatment processes are currently under development for the oxidation of trace organics, without the removal of dissolved solids. An example of such a system is shown on Figure 3(a). Another issue with DPR schemes employing reverse osmosis is the high energy usage required for treatment. An alternative treatment approach involves the use of electrodialysis as illustrated on Figure 3(b). New and enhanced biological treatment systems are also under development. As new technologies become available in the future, it is anticipated that constituent removal effectiveness will improve with a concomitant reduction in energy and resource usage.

## Integrated DPR treatment designs

The current trend in water and wastewater systems design can best be described as incrementalism. In examining the treatment process flow diagrams for DPR presented previously in Figures 2 and 3, it can be concluded that the production of purified water for DPR was an afterthought. Basically additional unit processes were tacked on to the end of existing secondary treatment process flow diagrams to remove specific compounds. However, at some point in the future there will need to be a complete rethinking of urban infrastructure to obtain the highest levels of performance and reliability. For water and wastewater systems, the advanced infrastructure model will likely include decentralization, remote management, resource recovery, source separated waste streams, and application of specific optimization of water quality. What is needed is the development of integrated water management systems in which new wastewater treatment plants are planned and designed from the ground up to optimize treatment performance with respect to the production of purified water, along with the recovery of energy and resources.

## SUMMARY

Because it is inevitable that DPR will become part of the water management portfolio for the reasons cited in this paper, it is important that water agencies begin to develop the necessary information that will allow DPR to become a reality. The technical feasibility of DPR is well established and will only get better in the future. In planning for wastewater treatment upgrades or

new plants that will be used to produce purified water, it is imperative that the incrementalism of the past be replaced with new integrated designs that will produce purified water along with the recovery of energy and resources.

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Review

# Groundwater recharge with reclaimed municipal wastewater: health and regulatory considerations

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**Abstract**

Groundwater recharge with reclaimed municipal wastewater presents a wide spectrum of technical and health challenges that must be carefully evaluated prior to undertaking a project. This review will provide a discussion of groundwater recharge and its management with special reference to health and regulatory aspects of groundwater recharge with reclaimed municipal wastewater. At present, some uncertainties with respect to health risk considerations have limited expanding use of reclaimed municipal wastewater for groundwater recharge, especially when a large portion of the groundwater contains reclaimed wastewater that may affect the domestic water supply.

The proposed State of California criteria for groundwater recharge are discussed as an illustration of a cautious approach. In addition, a summary is provided of the methodology used in developing the World Health Organization's *Guidelines for Drinking Water Quality* to illustrate how numerical guideline values are generated for contaminants that may be applicable to groundwater recharge.



*Keywords:* Advanced wastewater treatment; Drinking water guidelines; Groundwater recharge; Non-potable reuse; Pathogens; Organics; Potable reuse; Public health; Recharge; Soil-aquifer treatment; State of California; Wastewater reclamation and reuse; World Health Organization (WHO)

## 1. Introduction

Inadequate water supply and water quality deterioration represent serious contemporary concerns for municipalities, industries, agriculture, and the environment in many parts of the world. Factors contributing to these problems include continued population growth in urban areas, contamination of surface water and groundwater, uneven distribution of water resources, and frequent droughts caused by extreme global weather patterns. For more than a quarter century, a recurring thesis in environmental and water resources engineering has been that improved wastewater treatment provides a treated effluent of such quality that it should be put to beneficial use. This conviction in responsible engineering, coupled with increasing water shortages and environmental pollution, provides a realistic framework for considering reclaimed wastewater as a water resource rather than a liability.

Natural replenishment of underground water occurs very slowly; excessive exploitation and mining of groundwater at greater than the rate of replenishment causes declining groundwater levels in the long term and leads to eventual exhaustion of the groundwater resource. Artificial recharge of groundwater basins is becoming increasingly important in groundwater

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management and particularly where conjunctive use of surface water and groundwater resources is considered in the context of integrated water resources management.

Groundwater's major beneficial uses include municipal water supply, agricultural and landscape irrigation, and industrial water supply. The main purposes of artificial recharge of groundwater have been [1–3]: (a) to reduce, stop, or even reverse declines of groundwater levels, (b) to protect underground freshwater in coastal aquifers against saltwater intrusion and (c) to store surface water, including flood or other surplus water, and reclaimed municipal wastewater for future use. Groundwater recharge is also incidentally achieved in irrigation and land treatment and disposal of municipal and industrial wastewater via percolation and infiltration.

There are several advantages in storing water underground via groundwater recharge including:

(a) The cost of artificial recharge may be less than the cost of equivalent surface water reservoirs.
(b) The aquifer serves as an eventual natural distribution system and may reduce the need for transmission pipelines or canals for surface water.
(c) Water stored in surface reservoirs is subject to evaporation, taste and odor problems due to algae and other aquatic productivity, and to pollution, which may be avoided by soil-aquifer treatment (SAT) and underground storage.
(d) Suitable sites for surface water reservoirs may not be available or may not be environmentally acceptable.
(e) The inclusion of groundwater recharge in a wastewater reuse project may provide psychological and esthetic benefits as a result of the transition between reclaimed municipal wastewater and groundwater. This aspect is particularly significant when a possibility exists in the wastewater reclamation and reuse plans to augment substantial portions of domestic or drinking water supplies.

A wide spectrum of technical and health challenges must be carefully evaluated before undertaking a planned groundwater recharge project. Potential or hypothetical health risk considerations have limited expanding use of reclaimed municipal wastewater for groundwater recharge, when a large portion of groundwater contains reclaimed wastewater that may affect the domestic water supply.

Most of the research issues that address groundwater recharge and direct or indirect potable reuse are equally relevant to *unplanned* or *incidental* direct potable reuse such as municipal drinking water intakes located downstream from wastewater discharges or from polluted rivers and surface water reservoirs. Tapping of polluted water sources for unplanned or incidental potable reuse of polluted drinking water supply sources in the absence of adequate treatment may expose people to health risks not associated with protected water sources. Unresolved health concerns associated with drinking water drawn from polluted water sources certainly exist for wastewater reuse for potable purposes; however, a properly planned and managed water reuse project can produce higher quality finished water than unplanned reuse as is current common practice.

## 2. Techniques of groundwater recharge

Two types of groundwater recharge are commonly used with reclaimed municipal wastewater: surface spreading or percolation, and direct aquifer injection.

### 2.1. Groundwater recharge by surface spreading

Surface spreading is the simplest, oldest, and most widely applied method of artificial recharge [2]. In surface spreading, recharge waters such as treated municipal wastewater percolate from spreading basins through the unsaturated soil and ground (vadose) zone. Infiltration basins are the most favored methods of recharge because they allow efficient use of space and require only simple maintenance. In general, infiltration rates are highest where soil and vegetation are undisturbed.

Where hydrogeological conditions are favorable, wastewater reclamation can be implemented relatively simply by the SAT process. The necessary treatment can often be obtained by filtration as the wastewater percolates through the vadose zone, and then some distance laterally through the aquifer. Recommended pretreatment for municipal wastewater for the SAT process includes primary treatment or a stabilization pond, and dissolved air flotation. Pretreatment processes that leave high algal concentrations in the recharge water should be avoided, because algae can severely clog the soil of infiltration basins. While renovated wastewater from the SAT process is of much better water quality than the influent wastewater, it could be lower quality than the native groundwater. Thus, the SAT process should be designed and managed to avoid encroachment into the native groundwater and to use only a portion of the aquifer. The distance and transit time between infiltration basins and wells or drains should be as great as possible, usually at least 50–100 m and perhaps 6 months to give adequate SAT [1,4].

Advantages of groundwater recharge by surface spreading include: (a) groundwater supplies may be replenished in the vicinity of metropolitan and agricultural areas where groundwater over-drafting is severe, and (b) surface spreading provides the added benefits of

the treatment effect of soils and transporting facilities of aquifers.

### 2.2. Direct injection to groundwater aquifer

Direct subsurface recharge is achieved when water is placed directly into an aquifer. In direct injection, highly treated reclaimed water is pumped directly into the groundwater zone, usually into a well-confined aquifer. Groundwater recharge by direct injection is practiced: (a) where groundwater is deep or where the topography or existing land use makes surface spreading impractical or too expensive, and (b) when direct injection is particularly effective in creating freshwater barriers in coastal aquifers against intrusion of saltwater [1,2,4,5]. In arid climates where the practice of groundwater recharge is most imperative, recharge will occur through such means as dry riverbeds and spreading basins, and in most situations there will be an unsaturated zone between the surface and the aquifer.

Both in surface spreading and direct injection, locating the extraction wells as great a distance as possible from the spreading basins or the injection wells increases the flow path length and residence time of the recharged water. These separations in space and in time contribute to the mixing of the recharged water and the other aquifer contents, the opportunity for favorable biological and chemical transformations to occur, and to the loss of identity of the recharged water originating from municipal wastewater. The latter is an important consideration in successful reuse of treated wastewater in order to facilitate public acceptance.

## 3. Pretreatment for groundwater recharge

Four water quality factors are particularly significant in groundwater recharge with reclaimed wastewater: (a) microbiological quality, (b) total mineral content (total dissolved solids), (c) presence of heavy metal toxicants, and (d) the concentrations of stable and potentially harmful organic substances. Thus, groundwater recharge with reclaimed wastewater presents a wide spectrum of technical and health challenges that must be carefully evaluated. Some basic questions that affect pretreatment choices include [6–8]

- What treatment processes are available for producing water suitable for groundwater recharge?
- How do these processes perform in practice at specific sites?
- How does water quality change during infiltration–percolation and in the groundwater zone?
- What do infiltration–percolation and groundwater passage contribute to the overall treatment system performance and reliability?
- What are the important health issues to be resolved?
- How do these issues influence groundwater recharge regulations at the points of recharge and extraction?
- What benefits, problems, and successes have been experienced in practice?

Pretreatment requirements for groundwater recharge vary considerably depending upon the purpose of groundwater recharge, sources of reclaimed wastewater, recharge methods, location, and, more importantly, public acceptance. Although the surface spreading method of groundwater recharge is in itself an effective form of wastewater treatment, some level of pretreatment must be provided to municipal wastewater before it can be used for groundwater recharge. For direct injection of reclaimed municipal wastewater to groundwater aquifer where domestic water supply may be affected, an extensive treatment consisting of microfiltration and reverse osmosis and ultraviolet disinfection has been installed in several California groundwater recharge projects [5,9].

## 4. Health and regulatory aspects of groundwater recharge with reclaimed wastewater

It is essential that water extracted from a groundwater basin for domestic use be of acceptable physical, chemical, microbiological, and radiological quality. The main concerns are that adverse health risks could result from the introduction of pathogens or trace amounts of toxic chemicals into groundwater that is eventually to be consumed by the public. Every effort should be made to reduce the number of chemical species and concentrations of specific organic constituents in the applied water [8,10,11]. A source control program to limit potentially harmful constituents entering the wastewater collection system must also be an integral part of any groundwater recharge project. Extreme caution is warranted because of the difficulty in restoring a groundwater basin once it has been contaminated. In the USA, national/federal requirements for wastewater reclamation and reuse have not been established. As a consequence, water reclamation and reuse requirements for groundwater recharge are established by state agencies, e.g., the State of California Department of Health Services (DHS) and the Regional Water Quality Control Boards (RWQCBs) with a case-by-case determination for each project [11,12].

### 4.1. Health considerations in groundwater recharge with reclaimed wastewater

Groundwater recharge with reclaimed municipal wastewater share many of the public health concerns

encountered in drinking water withdrawn from polluted rivers and surface water reservoirs. The ramifications of long-term exposure to many of the chemical constituents in trace quantities are not well understood although the risks, if any, should be very low in well-treated recharged groundwater, and probably no greater than for typical surface water. Nevertheless, regulatory agencies are proceeding with extreme caution in permitting water reuse applications that affect potable water supplies [11,13].

Because of health and aesthetic concerns, drinking water is the highest level end use with the most stringent water quality requirements. The World Health Organization's (WHO) *Guidelines for Drinking Water Quality* (GDWQ) and the methodologies described therein provide a good initial basis for evaluating the quality and safety for consumption of drinking water, but they alone were not intended to be applied to drinking water derived from significantly contaminated sources. The GDWQ and the US National Drinking Water Regulations typically address source water derived from lakes, wells and rivers, which, although frequently contaminated, are almost always of much better quality and more diluted than municipal wastewater. Thus, guidelines and standards assume source water that would not contain significant quantities of known or unknown hazardous contaminants, and waters that have had a long history of apparently safe use albeit after suitable water treatment has been applied.

The irony is that water derived from the 'natural' but obviously imperfect sources, often receives only basic treatment (filtration and disinfection). The final product might not be as high quality as the reclaimed wastewater that has been subjected to much more rigorous treatment, water quality control, and management. The strengths of planned wastewater reclamation and groundwater recharge are that those projects are designed specifically to address the challenges associated with contaminated sources. They are designed, monitored and managed to assure that potential risks are consciously controlled. There is an extra burden to demonstrate that the source water, as proposed to be treated, managed and stored, will be appropriate for the intended use, and will not bear an unacceptable risk for the users.

Each proposed groundwater recharge project should be assessed with respect to the types and quantities of contamination in the source water (e.g., containing industrial and/or domestic wastewater, and unique contaminants). Other factors include the degree of pretreatment and the quality prior to surface spreading or injection into groundwater aquifers, the length of storage time and passage distance which can attenuate contaminants in the ground, the degree of dilution with groundwater, and the type, capability and reliability of treatment that the water will receive when extracted, and finally the extent and type of human exposure to result from the end use; e.g., ingestion, inhalation of aerosols, and dermal exposure, even when potable reuse is not intended.

Pathogenic microorganisms are by far the predominant concern, but trace chemicals must also be considered. Measurement techniques are available for virtually all inorganic substances and radionuclides. Well-established risk assessment methods exist for determining acceptable concentrations below which there is no significant risk to humans. However, some organic constituents are more difficult to assess [14,15].

To form a protective policy, the following questions should be considered: (a) is a water reuse option necessary as a water resource alternative; (b) what level of risk control is attained by a standard relative to the intended use; (c) how valid is the judgment of that level of risk, and, what is the acceptability of a given degree of risk? Risk analysis as applied to natural or reclaimed water entails the same difficulties as that for other health hazards in the environment. Basically, the problem lies in attempting to estimate the hypothetical risks involved and agreeing upon what level of risk to accept [16].

### *4.2. Concerns for pathogens, trace organics, and public health*

Control of viruses and protozoa in reclaimed wastewater is of paramount concern even though such product water may meet microbiological standards set for drinking water, e.g., less than or equal to one total coliform bacterium/100 ml, or no detectable *E. coli* per 100 ml. The principal reason is that reclaimed wastewater is derived directly from municipal wastewater in which pathogen concentrations are higher than even heavily polluted natural waters, and the typical microbiological indicators alone are inadequate for that application. Thus, more extensive regimens for controlling and monitoring of microbial agents must be applied, and additional standards are required. Because routine monitoring for pathogens is not feasible, expensive and not real time; it is more important to design multiple-barrier systems to assure continuous production of safe water.

Removal of specific trace organic compounds through full-scale advanced wastewater treatment (AWT) processes including chemical clarification, filtration, air stripping, activated carbon adsorption, microfiltration (MF), nanofiltration (NF), reverse osmosis (RO), and advanced oxidation using hydrogen peroxide and UV irradiation has been demonstrated. These studies show that there is the capability to control virtually all synthetic organic compounds (SOC) to below current limits of acceptability. However, the majority of higher molecular weight "natural" organic compounds in AWT effluents were unidentified and of generally

unknown health significance. Recently, however, methodologies have been developed to identify or classify most of the NOM. The presence of natural organic matter (NOM) also contributes to the formation of disinfection byproducts (DBPs) including trihalomethanes (THM) and other organic halogens (TOX) of potential health significance. The often observed mutagenic activity of AWT effluents is of unknown health significance and a matter of continuing research interest.

Emerging contaminants relevant to groundwater recharge will include: (a) trace organics such as: potential endocrine disrupting compounds (EDCs), pharmaceutically active compounds (PhACs), and *N*-nitrosdimethylamine (NDMA), (b) some trace inorganics and (c) microbes, e.g., nanobacteria ($\approx 0.1\,\mu m$). Wastewater indicators, EDCs, and PhACs selected for study usually are not detected in either NF or RO permeates at pilot- and full-scale. These findings indicate that advanced membrane treatment using NF or RO not only efficiently removes high molecular weight organic carbon compounds, but also selected organic wastewater indicators, such as EDCs and PhACs [17].

### 4.3. Rationale for establishing groundwater recharge guidelines and regulations

Risk avoidance or risk minimization certainly should be principal elements in the determination of recharge water standards and guidelines in relation to their end uses. However, technological and economic factors also enter into the ultimate quality parameters. Aesthetic factors of taste, odor, and appearance must be important considerations for drinking water even if they do not directly relate to the safety of the water, because consumer acceptance and confidence in the quality and safety are essential.

### 4.4. Risk assessment for water intended for human consumption

Risk assessment is fundamentally an attempt to quantify the possible health consequences of human exposure in particular circumstances. In the case of drinking water the conclusion would be expressed in terms of the probability (within specified levels of uncertainty) of cases of adverse effects (e.g., fatalities) in the reference population group; for example, an incremental upper bound risk of bladder cancer of one/million (E-6) in a population typically consuming 2 L of drinking water per day for 70 years. The lower bound risk might well be zero, especially if one or more assumptions is invalid. All of these computations and *conclusions* are limited in their reliability and credibility by the quality of the exposure and toxicological data, the mathematical expressions used, and the lack of scientific understanding of the mechanisms of carcinogenesis operative at low environmental doses in genetically diverse humans, as opposed to the high doses to which test animals are exposed. In addition, the significance of low-dose interactions between chemicals is a virtual unknown [18].

In its lowest terms a risk assessment (RA) could be represented as follows:

$$\begin{aligned} \mathrm{RA} = {} & (\text{concentration distribution}) \\ & \times (\text{persons exposed at each dose}) \\ & \times (\text{risk per dose}) \times (\text{time}). \end{aligned} \quad (1)$$

The basic information required to perform a qualitative and quantitative risk assessment includes quantitative information on: (a) the occurrence, (b) human exposure, and (c) toxicology of the substance. Although methodologies are available to attempt to quantify each of these factors, in practice, data limitations and analytic complexities usually lead to many simplifying assumptions.

Computing human exposure from occurrence data requires detailed information on water and food consumption patterns and other life-style factors that often are very difficult to model. These would be age-, size-, season-, and location-dependent. Water consumption has been studied in several countries and reasonable distributional data are available. For example, the average drinking water consumption estimated from eight studies was 1.63 L/d. A dietary study [19] concluded that the median daily water consumption in the USA was 1.2–1.4 L/d and that 80–85% consumed less than 2 L, and about 1% consumed more than 4 L per day. This included all tap water including coffee, tea, and reconstituted juices, soups, and food water (e.g., from rice). These estimates are probably low for very warm climates.

Dietary patterns are, however, much more complex and databases amenable to extrapolation to populations are not very extensive. Localized ambient air inhalation data are available for a few substances. Indoor air quality data are potentially of greatest interest but also limited. Water can also contribute to indoor air exposure to volatile substances such as trihalomethanes or radon, or even *Legionella* spp. organisms from growth in plumbing systems. This indirect exposure should also be considered when projecting total exposure and the drinking water contribution. For VOCs in drinking water, this inhalation dose can be equivalent to the amount from ingestion of water. Drinking water standards and guidelines usually have large safety margins that accommodate the inhalation contribution to total intake.

A conceptual framework for various assays and the relative significance to human health is shown in Fig. 1. Toxicological risks that are postulated for exposure

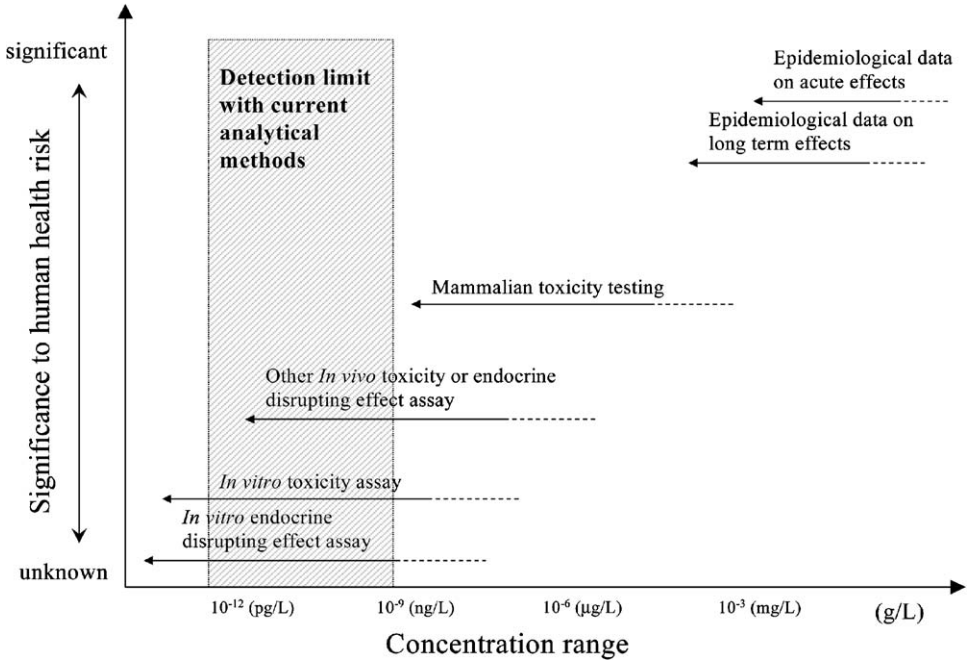


Fig. 1. Conceptual framework for various assays for trace organic compounds and their relative significance to human health risk (Adapted from Tsuchihashi et al. [20]).

levels typical to drinking water are usually well beyond the capability of epidemiological studies to measure. Since regulatory policy generally strives to limit risks nominally below about 1/100,000 for life threatening diseases like cancer, these lower risks are projected orders of magnitude beyond the experimental data by making inferences about the shape of the dose–response curve and extrapolations from effects to humans at higher doses or animal testing, and in vitro assays. At times these projections may encompass a million-fold range with commensurate uncertainty. Imperfect though this system is, it attempts to incorporate all of the available information and creating usable (albeit unverifiable) low dose risk hypotheses that can be helpful for decision making that is designed to err on the side of safety. Thus, *WHO Guidelines for Drinking Water Quality* [21,22] along with expanded detection and evaluation methodologies aimed at the source specific contaminants, and site and technology specific factors should be applied on a case-by-case basis and extended significantly to determine the design and operation of each specific project to assure the suitability of the product water for its end use. Expansion of these guidelines to include wastewater reclamation and recharge applications is recommended. Indeed, the recommendations and methodologies described in the *WHO Guidelines for Drinking Water Quality* provide for appropriate authorities to make suitable water quality and safety determinations based upon the societal, economic and feasibility factors that bear upon the cost/risk/benefit balance that must be struck to assure access to water of both adequate quantity and quality.

A brief description of the type of process used by WHO and US regulatory agencies to determine acceptable concentrations of contaminants in drinking water is provided in Appendix A [23]. The methodology is evolving and variations are commonly applied, but this does describe the basic thought processes that are involved. The methodologies may also be applicable to groundwater recharge with reclaimed municipal wastewater.

## 5. Proposed State of California groundwater recharge criteria

The proposed California criteria for groundwater recharge with reclaimed municipal wastewater rightly reflect a cautious attitude as discussed above toward short-term and long-term health concerns. The criteria rely on a combination of controls intended to maintain a microbiologically and chemically safe groundwater recharge operation. No single method of control would be effective in controlling the transmission and transport of contaminants of concern into and through the environment. Therefore, source control, wastewater

treatment processes, water quality, recharge methods, recharge area, dilution, extraction well proximity, and monitoring wells are all specified. An illustration of this cautious and conservative approach for regulating planned groundwater recharge projects is given in Appendix B, excerpted from draft DHS regulations dated April 23, 2001 [24,26].

California's groundwater recharge criteria are not necessarily applicable to circumstances with different water quantity/quality, economic and risk/benefit environments, but they are instructive of the potential for a comprehensive and protective regulatory program being implemented. These proposed groundwater recharge criteria have undergone several iterations since the early 1990s, and, while several refinements have been made to improve the criteria, many of the requirements specified in earlier drafts remain unchanged. More recent revisions emphasized dilution and unregulated organics and groundwater mound monitoring.

## 6. Summary and conclusions

To increase the supply of groundwater, artificial recharge of groundwater basins is becoming increasingly important in groundwater management and particularly where the conjunctive use of surface water and groundwater resources is planned. Use of reclaimed wastewater including groundwater recharge for a variety of applications has been implemented and it is safely undertaken provided appropriate planning, treatment, water quality control, assessment, and precautions are followed.

The lack of specific criteria and guidelines governing artificial recharge of groundwater is currently hampering the implementation of additional large-scale groundwater recharge operations. Thus, the establishment of policies and guidance for planning and implementing new groundwater recharge projects is encouraged. The rational basis and other background information for producing groundwater recharge guidelines were briefly presented in this paper and in key references and are further elaborated in Appendices A and B.

Drinking water will be the highest level use with the most stringent quality requirements. The WHO's *Guidelines for Drinking Water Quality* and the methodologies described therein provide a good initial basis for evaluating the quality and safety for consumption of drinking water from common sources, but they alone were not intended to be applied to drinking water derived from significantly contaminated sources such as municipal wastewater. The State of California's draft groundwater recharge criteria is also presented emphasizing a multiple barrier approach.

Much of the concerns and research that address groundwater recharge and potable water reuse are of equal relevance to unplanned or incidental direct potable reuse such as the common practice of municipal drinking water supply intakes located downstream from wastewater discharges or from increasingly polluted rivers and surface water impoundments. The strengths of planned water reuse and recharge are that those projects are designed specifically to address the challenges associated with contaminated sources. They are designed, monitored and managed to assure that the potential risks are consciously controlled.

Chemical and microbial contamination, hazards and risks as well as aesthetic characteristics are the key decision factors for a proposed use of a water of a particular quality. Measurement techniques are available for virtually all inorganic substances and natural and synthetic radionuclides. Well-established risk assessment methods exist for determining acceptable concentrations below which there is no significant risk to humans.

Microbial contaminants can be bacterial, viral, or protozoan or larger organisms and they are by far the most important common risk factors when producing drinking water or reclaimed water for direct or indirect human contact. Their control should never be compromised because of other treatment considerations, e.g. disinfectant byproducts.

The most controversial group of chemical contaminants in wastewaters is the organic substances, which are always present and most difficult to measure and assess. These are mostly natural products, most of which are not likely to be harmful as well as industrial chemicals, and disinfection byproducts. Most discreet industrial chemicals and many disinfection byproducts are measurable by sophisticated instrumental methods, and procedures are available to assess exposure risks in many cases; however, often insufficient experimental toxicology data are available to perform detailed risk assessments. Most of the primarily natural organic chemicals and their derivatives have been historically not readily identifiable; however, great progress is now being made in their characterization. In general, operational standards for water reuse projects have tended to rely on use of treatment trains designed to give significant reduction of difficult to define organic chemicals using a non-specific chemical indicator such as TOC along with measurements and criteria for specific chemicals, (e.g., benzene or nitrosamines). This approach can show that a large portion of the organic chemicals of most types has been removed by the treatment technology, and in addition that specific measurable hazardous chemicals do not exceed limits. If the final TOC is low enough then it is logical that insignificant amounts, if any, of the difficult to define substances of unknown concern remain.

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## Appendix A. WHO Guidelines For Chemical Drinking Water Quality

### *A.1. Introduction*

The primary aim of the Guidelines for Drinking Water quality is the protection of public health [21,22]. The latest iteration of the WHO Guidelines is being prepared for release in late 2004. These health-based guidelines are intended to be used as a basis for the development of national standards that, if properly implemented, will ensure the safety of drinking water supplies through the elimination, or reduction to a minimum concentration, of constituents of water that are known to be hazardous to health. Guideline values are not mandatory limits. Thus, the guideline values must be considered in the context of local or national environmental, social, economic, and cultural conditions. The main reason for not providing international standards for drinking water quality is the advantage provided by the use of a risk-benefit approach (qualitative or quantitative) to the establishment of national standards and regulations. The guideline values have sufficient flexibility (i.e. acceptable ranges) to enable national authorities to make judgments regarding the specific values to be required for drinking water of acceptable quality and safety.

Most problems associated with chemical constituents of drinking water arise primarily from their hypothetical potential to cause adverse health effects after prolonged periods of low dose exposure. Of particular concern are contaminants that have cumulative toxic properties, such as heavy metals, and carcinogenic substances. Few common chemical constituents of water can lead to acute health problems except through massive accidental or deliberate contamination.

Guideline values have been set for numerous potentially hazardous water constituents and provide a basis for assessing drinking water quality. They represent the concentration of a constituent that would not, with a margin of safety, result in any significant risk to the health of the consumer over a lifetime of consumption. Guideline values are not set at concentrations lower than the detection limits achievable under routine laboratory operating conditions. Moreover, guideline values are recommended only when control techniques are available to remove or reduce the concentration of the contaminant to the desired level.

In some instances, provisional guideline values have been set for constituents for which (a) there is some evidence of a potential hazard but where the available information on health effects is limited, or (b) the calculated guideline value would be below the practical quantification level, or below the level that can be achieved through practical treatment methods.

### *A.2. Assumptions*

(a) *Drinking water consumption and body weight*. In developing the guideline values for potentially hazardous chemicals, a daily per capita consumption of 2 L by a person weighing 60 kg was generally assumed. The guideline values set for drinking water using this assumption do, on average, err on the side of caution. However, such an assumption may underestimate the consumption of water per unit weight, and thus exposure, for those living in hot climates as well as for infants and children who consume more fluid per unit weight than adults.

(b) *Inhalation and dermal absorption*. The contribution of drinking water to daily exposure includes direct ingestion as well as some indirect routes, such as inhalation of volatile substances and dermal contact during bathing and showering. That portion of the total tolerable daily intake (TDI) allocated to drinking water is generally sufficient to allow for these additional routes of intake.

(c) *Mixtures*. Chemical contaminants of drinking water supplies are present together with numerous other organic and inorganic constituents. The guideline values were calculated separately for individual substances; the large margin of safety incorporated in the majority of guideline values is considered to be sufficient to account for potential interactions of each substance with other compounds present.

(d) *Health risk assessment*. The principal sources of information on health effects resulting from exposure to chemicals used in deriving guideline values are human epidemiology and animal toxicology. Epidemiology is usually limited due to lack of quantitative information on the concentrations to which people are exposed or on simultaneous exposure to other agents, and because the epidemiological tools are relatively insensitive to low risk situations due to confounders. Animal studies are generally limited because of the small number of animals used and the high doses administered, as well as the need

to extrapolate the results to the lower doses to which human populations are exposed.

(e) *Derivation of guideline values using a TDI approach*. For most kinds of non-cancer toxicity, it is generally believed that there is a dose to individuals below which no adverse effects will occur. For chemicals that give rise to such toxic effects, a TDI can be derived as follows:

$$\mathrm{TDI} = \frac{\mathrm{NOAEL\ or\ LOAEL}}{\mathrm{UF}},$$

where NOAEL is the no-observed-adverse-effect level, LOAEL the lowest-observed-adverse-effect level, UF the uncertainty factor.

The guideline value (GV) is then derived from the TDI as follows:

$$\mathrm{GV} = \frac{\mathrm{TDI} \times \mathrm{bw} \times P}{C},$$

where, bw is the body weight (60 kg for adults, 10 kg for children and 5 kg for infants), $P$ the fraction of the TDI allocated to drinking water, $C$ the daily drinking water consumption (2 L for adults, 1 L for children, 0.75 L for infants).

(f) *Tolerable daily intake*. The TDI is an estimate of the total amount of substance in food or drinking water, expressed on a body weight basis (mg/kg or μg/kg of body weight), that can be ingested daily over a lifetime without appreciable health risk.

Short-term exposure to levels exceeding the TDI is not a cause for concern, provided the individual's intake averaged over longer periods of time does not appreciably exceed the level set. However, consideration should be given to any potential acute toxic effects that may occur if the TDI is substantially exceeded for short periods of time.

(g) *Uncertainty factors*. There were four sources of uncertainty, each assigned a factor of 1–10: interspecies variation (animals to humans), intraspecies variation (individiual variations), adequacy of studies or database, and nature and severity of effect. For most contaminants, there is great scientific uncertainty, and hence, there may be a large margin of safety above the guideline value before adverse health effects might result.

(h) *Allocation of intake*. In many cases, the intake of a substance from drinking water is small in comparison with that from other sources such as food and air. Guideline values derived using the TDI approach take into account exposure from all sources by apportioning a default percentage (commonly 10%) of the TDI to drinking water. This conservative approach ensures that the total daily intake from all sources does not exceed the TDI.

(i) *Derivation of guideline values for potential carcinogens*. Evaluation of the potential carcinogenicity of chemical substances is usually based on long-term animal studies. Sometimes data are available on carcinogenicity in humans, mostly from occupational exposure. On the basis of the available toxicological evidence, the International Agency for Research on Cancer (IARC) categorizes chemical substances with respect to their potential to be carcinogenic to humans.

It is generally considered that the genotoxic mechanism of chemical carcinogenesis does not have a threshold; consequently, there is a probability of harm at any level of exposure albeit vanishingly small at extremely low levels. Therefore, the development of a TDI is considered inappropriate, and probabilistic low-dose risk extrapolation is applied. The linearized multistage model was generally adopted in the development of the guidelines, and the guideline values are presented as the concentration in drinking water associated with an estimated excess lifetime cancer risk of $10^{-5}$ ($\pm$a factor of 10) from consumption of 2 L of water per day. These models provide, at best, a rough projection of the cancer risk; they do not usually take into account a number of biologically important considerations, such as detoxification pathways, pharmacokinetics, DNA repair, or immunological protection mechanisms. The models used are conservative and probably err on the side of caution.

Some carcinogens are capable of producing tumors in animals or humans without exerting genotoxic activity, but acting through an indirect mechanism. It is generally believed that a threshold dose exists for these non-genotoxic carcinogens, and guideline values for these compounds were calculated using the TDI approach.

## Appendix B. Summary of proposed State of California criteria for groundwater recharge

Summary of proposed groundwater recharge criteria with reclaimed municipal wastewater is shown in Table 1.

*B.1. Source control*

A well operated and strictly enforced source control program is a prerequisite to groundwater recharge project which must be approved by the State of California Regional Water Quality Control Boards.

*B.2. Treatment processes*

The definition of "filtered disinfected wastewater" in the proposed revisions to the existing regulations for nonpotable uses of reclaimed wastewater now includes the use of membranes to meet the filtration requirements. This includes and does not distinguish between microfiltration, ultrafiltration, nanofiltration, and reverse osmosis. Although the performance requirement for membranes is more stringent than that for granular medium filtration (average 0.2 NTU versus 2 NTU), the work done by the City of San Diego, California indicates that a filtered wastewater turbidity of greater than 0.1 NTU signals a breach in the integrity of the membranes.

Table 1
Proposed State of California criteria for groundwater recharge with reclaimed wastewater

| Contaminant type | Type of recharge | |
|---|---|---|
| | Surface spreading | Subsurface injection |
| Pathogenic microorganisms | | |
| Secondary treatment | SS $\leqslant$30 mg/L | |
| Filtration–turbidity | $\leqslant$2 NTU | |
| Disinfection | 4-log virus inactivation, $\leqslant$2.2 total coliform 100 mL | |
| Retention time underground | 6 mos. | 12 mos. |
| Horizontal separation | 153 m | 610 m |
| Regulated contaminants | Meet all drinking water maximum contaminant levels (MCLs) | |
| Unregulated contaminants | | |
| Secondary treatment | BOD $\leqslant$30 mg/L, TOC $\leqslant$16 mg/L | |
| Reverse osmosis | Four options available depending on local conditions | 100% treatment to TOC $\leqslant \frac{1\ \text{mgTOC/L}}{\text{RWC}}$ |
| Spreading criteria for SAT 50% TOC Removal credit | Depth to groundwater at initial percolation rates of: $<$0.5 cm/min = 3 m. $<$0.8 cm/min = 6 m. | NA |
| Mound monitoring option | Demonstrate feasibility of the mound compliance point | NA |
| Recycled water contribution | $\leqslant$50% of affected groundwater volume | |

Note: RWC = the percent recycled water contribution in groundwater extracted by drinking well water. Adapted from State of California [25], Crook et al. [24], and Hultquist et al. [26].

Also included in the definition of filtered disinfected wastewater is the requirement that the wastewater be oxidized to a TOC concentration of 16 mg/L or less. The current State of California's *Wastewater Recycling Criteria* defines "oxidized wastewater" as wastewater in which the organic matter has been stabilized, is nonputrescible, and contains dissolved oxygen. The TOC requirement of 16 mg/L is a performance based water quality standard.

To address the issue of unregulated organics, the previous drafts of the proposed criteria allowed the use of granular activated carbon (GAC) or reverse osmosis (RO) for organics removal. While it was recognized that GAC and RO could be complementary with respect to the fractions of organics removed by the processes, GAC is generally regarded as not being as efficient as RO for organics removal. Consequently, the proposed groundwater recharge regulations reflect the conclusion that GAC alone is not deemed to be an effective process for controlling unregulated organics.

*B.3. Disinfection*

The disinfection requirement in the proposed California regulations for non-potable reuse where a high degree of public exposure is expected is also required for all groundwater recharge projects. This is because it assures a substantial log virus reduction, which is the only pathogenic microorganism not effectively removed by the aquifer. Many groundwater recharge projects also provide non-potable water for other urban uses, and the disinfection requirement is readily achievable with reclamation technologies commonly in use in California. The two options for compliance are: (a) filtration followed by chlorination with a modal chlorine contact time multiplied by the chlorine residual (CT value) of 450 mg-min/L; or (b) any combination of filtration and disinfection that has been demonstrated, and is operated, to achieve a 5-log virus reduction.

*B.4. Water quality*

While the application of an organics removal requirement would appear to solve a plethora of water quality issues, several water quality issues remain. For example, the nitrogen requirement remains under discussion. A proposed total nitrogen standard of 10 mg-N/L was developed in conservative manner to ensure that, should all ammonia forms of nitrogen be converted to nitrate, the effluent nitrate concentration would approach, but never exceed the nitrate maximum contaminant level (MCL). Dilution underground is not considered to be a reliable method for controlling the nitrogen content of the water for a chemical that poses such acute public health threat. Therefore, the total nitrogen standard must be met above ground.

At issue is the nitrite drinking water MCL of 1 mg-N/L. Since biological nitrification and denitrification processes produce nitrite as an intermediate product, it is not known how protective the 10 mg-N/L standard would be of the nitrite MCL.

*B.5. Dilution and unregulated organics*

The draft criteria use the percent of the drinking water supply that comes from recycled municipal wastewater

as a factor in determining the required degree of unregulated organic removal. This fraction is the recycled water contribution (RWC). The previous drafts set separate organic chemical removal requirements for subsurface injection and surface spreading projects going to a 20% RWC and those going to a 50% RWC.

The proposed criteria now contain one set of requirements (in a continuum) for projects with a recycled water concentration up to 50%. Although there are provisions for allowing up to a 100% RWC, the criteria establish, in effect, a dilution requirement for most groundwater recharge reuse projects. The rationale for maintaining this dilution requirement has not changed. An alternative to the 50% maximum RWC criterion is proposed that will assure an equal level of public health protection [24,25].

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