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Reclaimed wastewater: preventing viral pathogens on plant foods

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Climatic changes are increasing the pressure on water resources in Germany and Europe. To counter this pressure, the use of reclaimed wastewater for agricultural irrigation has legally been made possible at the European level. Different methods of wastewater treatment are available. Uniform minimum requirements for water reuse are intended to ensure human and animal health as well as environmental protection. The presence of viral pathogens in wastewater poses a major challenge in this context. Against this background, the German Federal Institute for Risk Assessment (BfR) has evaluated scientific literature on the health risk of using reclaimed wastewater for the irrigation of plants used as food with regard to viral pathogens. The particular focus is on fruit and vegetables that are consumed raw, where possible pathogens are not reduced or killed by heating.

In particular, the human norovirus, which causes gastrointestinal illness, and the hepatitis A virus, which can lead to liver inflammation, are viruses that can be transmitted via plant foods. For both viruses, numerous disease outbreaks have been described, which can be traced back to the consumption of plant foods such as frozen berries and leafy vegetables. Additionally, other viruses such as the hepatitis E virus, rotavirus, sapovirus, astrovirus and adenovirus also play a role. In general, the severity of the triggered disease can vary depending on the virus and the health condition of the affected person.

A current insufficient data situation makes a conclusive risk assessment of the use of reclaimed wastewater for the irrigation of fruit and vegetables with regard to pathogenic viruses difficult. There are currently few data on the stability and inactivation of human norovirus and hepatitis A virus, including their behaviour during wastewater treatment, in soil, on plant foods and on the uptake of these viruses into the plant via the roots. However, the available data and studies with closely related viruses show that in most cases the viruses have a very high stability to environmental influences, in the soil and on the plant, and can be taken up via the roots. In addition, most of the relevant viruses have a very low minimum infective dose, which means that even small amounts of virus can lead to disease. In contrast, high amounts of viruses are excreted in the stool, which in turn suggests a high load in the wastewater.

In addition to the data gaps identified, there is also a need for research on methods to investigate the effectiveness of inactivation of relevant viruses by different wastewater treatment systems and the quality of the reclaimed wastewater with regard to the presence of infectious viruses.

Even if the current data situation is still insufficient, it is recommended in the interest of consumer health protection to refrain from irrigating plants with reclaimed wastewater, whose parts growing close to or in the ground are usually consumed raw. This recommendation applies until appropriate treatment procedures and controls can ensure that no pathogenic viruses are present in the irrigation water. In the case of plants that are not consumed raw, adverse health effects from irrigation with reclaimed wastewater are not to be expected according to current knowledge, as long as the food is sufficiently heated before consumption.

BfR	BfR risk profile: Infection with norovirus and hepatitis A virus after consumption of raw fruit and vegetables irrigated with reclaimed wastewater (opinion 019/2022)				
A Affected persons	**General population Senior citizens Chronically ill**				
B Likelihood of impairment to health from consumption of raw fruit and vegetables irrigated with reclaimed wastewater	Practically impossible	Unlikely	Possible	Probable	Certain
C Severity of impairment to health from consumption of raw fruit and vegetables irrigated with reclaimed wastewater	No impairment (only general population)	Mild impairment [reversible]	Moderate impairment [reversible]	Severe impairment [irreversible] (only senior citizens and chronically ill)	
D Validity of available data	High: The most important data are available and are internally consistent	Medium: Some important data are missing or contradictory	Low: A large volume of important data is missing or inconsistent		
E Controllability by the consumer	Controls not needed	Controllable with precautionary measures	Controllable by avoidance	Not controllable	

Explanations

The risk profiles are intended to visualise the risk outlined in the Opinion. The profile is not intended to be used to compare risks. The risk profiles should only be read in conjunction with the Opinion.

[1] Line B – Likelihood of impairment to health
The probability of an adverse health effect is influenced, among other things, by the quantity of pathogens present in the irrigation water, the type of plants irrigated and the irrigation technique. The BfR expects this probability to increase if plants, whose parts growing close to or in the ground are intended for raw consumption, are irrigated with reclaimed wastewater in Germany.

[2] Line C - Severity of impairment to health
The severity of the impairment can vary depending on the immune status, age and previous illnesses. Particularly in very old people, moderately severe health impairments and occasional deaths are possible with regard to the norovirus and the hepatitis A virus. In persons with liver damage, moderately severe health impairments and occasional deaths are also possible with regard to the hepatitis A virus.

[3] Line D – Validity of available data
Insufficient data and the lack of suitable detection methods for norovirus and hepatitis A virus in reclaimed wastewater make a conclusive risk assessment of the use of reclaimed wastewater for the irrigation of fruit and vegetables with regard to norovirus and hepatitis A virus difficult.

[4] Line E - Controllability by the consumer
Because of the low infective dose, consumers have no means of controlling the risk apart from heating fruit and vegetables completely before consumption. It is therefore all the more important that no human pathogenic viruses are present in the irrigation water of plant foods that are consumed raw. With regard to the hepatitis A virus only, consumers have the possibility to control the risk by vaccination.

1 Subject of the assessment

Regulation (EU) 2020/741 on minimum requirements for water reuse entered into force on 26 June 2020 and will become applicable on 26 June 2023. Numerous questions still need to be clarified for the national implementation of this regulation. Safe use of reclaimed wastewater in agricultural irrigation requires, among other things, that no adverse health effects for consumers are to be expected. For this reason, the present opinion provides an initial assessment of the health risk posed by the use of reclaimed wastewater for the irrigation of plants that are to be used as foodstuffs, with regard to illness-causing (human pathogenic) viruses. It was further examined whether restrictions on the use of reclaimed wastewater, additional validation parameters for wastewater treatment or additional controls of the reclaimed wastewater with regard to hazards from human pathogenic viruses are necessary.

2 Results

After evaluating publications, the BfR concludes that a final risk assessment for the use of reclaimed wastewater for the irrigation of plants with regard to human pathogenic viruses is currently not possible due to a lack of data. However, the limited data available suggest that transmission of human pathogenic viruses to humans via irrigation of plants with contaminated reclaimed wastewater and their subsequent consumption is possible and highly plausible. Therefore, strict limits should apply to the use of reclaimed wastewater for irrigation with respect to plant foods to be consumed raw.

Viruses that can be transmitted via plant foods include, above all, the human norovirus, which causes gastrointestinal disease, and the hepatitis A virus, which can lead to liver inflammation. Furthermore, other viruses that can be transmitted via the gastrointestinal tract (enterally), such as hepatitis E virus, rotavirus, sapovirus, astrovirus and adenovirus can play a role in this context. The severity of the illnesses caused varies depending on the type of virus and patient group, with hepatitis pathogens frequently leading to hospitalisations and gastroenteritis pathogens frequently being associated with high case numbers. For human noroviruses and hepatitis A viruses, numerous outbreaks have been described that were caused by the consumption of plant foods such as frozen berries and leafy vegetables.

Human noroviruses cannot yet be efficiently propagated in cell cultures, which is why the determination of their infectivity in a sample is very difficult. Therefore, data on the stability and inactivation of human noroviruses are largely lacking. Numerous stability and inactivation studies have been conducted using closely related viruses - so-called surrogate viruses. However, the behaviour of these viruses is not identical to that of human noroviruses, which is why the validity of such studies for the behaviour of human noroviruses is questionable. A similar situation exists for the hepatitis A virus, for which only very few cell culture-adapted strains exist. Many studies on the stability and inactivation of hepatitis A virus are therefore based on data from a single strain, which is why the diversity of the actually occurring field strains cannot be represented. For the reasons mentioned above, very few data currently exist on the stability and inactivation of human norovirus and hepatitis A virus during wastewater treatment, in soil, on plant foods and on the uptake of these viruses via the roots into plants.

When considering the few available data for the human pathogenic viruses as well as the available data of the surrogate viruses examined, however, it can be stated that the viruses in most cases show a very high stability towards different physico-chemical parameters, in the soil and on the plant. Uptake (internalisation) into the plant via the roots has been shown in most studies, at least based on the detection of viral ribonucleic acid (RNA). RNA of the viruses of interest has been detected on plant foods in several studies and a large number of viral disease outbreaks following consumption of plant foods have been documented. The diseases triggered have a high relevance for the health of the population. An exact estimation of the transmission risk is not possible. However, most of the viruses of interest have a very low minimum infective dose, which means that even small amounts of virus after ingestion can lead to disease. On the other hand, high amounts of viruses are excreted in the stool, which suggests the possibility of high contamination of wastewater. In addition, for the following reasons, it cannot be ensured that the virus infectivity is sufficiently reduced by wastewater treatment in accordance with Regulation (EU) 2020/741 and that the viruses are sufficiently inactivated in the soil and on or in the plant. Against this background, the risk characterisation for different methods of irrigation was therefore based on a "worst case" scenario.

Based on the results of the risk characterisation carried out, the BfR recommends not using reclaimed wastewater for the irrigation of plants, whose parts growing close to or in the ground are intended for raw consumption. This recommendation applies until it can be ensured by suitable treatment processes and controls that no human pathogenic viruses are present in the irrigation water. According to the current state of knowledge, infectious human pathogenic viruses present in the reclaimed wastewater can get onto or into the edible parts of the plants in any of the irrigation systems considered (subsurface drip irrigation, drip irrigation, furrow irrigation, sprinkler system, hydroponic culture[1]) and cause illness in humans when consumed raw.

When comparing the different irrigation systems, subsurface drip irrigation and hydroponic culture appear to be associated with the lowest probability of virus contamination of aboveground parts of the plant. However, there is a lack of data on the uptake of human pathogenic viruses via the roots into the plant and their subsequent infectivity in the aboveground part of the plant, although some data obtained on surrogate viruses suggest such uptake. Therefore this type of irrigation with reclaimed wastewater cannot be recommended either.

No data are currently available on viruses for the evaluation of irrigation of fruit trees and vineyards with reclaimed wastewater. However, there are also no indications of disease outbreaks caused by viruses after the consumption of fruits grown on trees or grapes. An efficient uptake of viruses via the roots of fruit trees and vines with subsequent transport over relatively long distances into the fruit seems rather unlikely according to the current state of science, which is why adverse health effects are not to be expected from irrigating these plants with reclaimed wastewater (minimum quality class A or B). However, this assessment only applies if no irrigation takes place during which the fruits can come into direct contact with the reclaimed wastewater.

For the irrigation of fodder crops, no concrete health risks arise for the viruses mainly considered here (human norovirus and hepatitis A virus), as these viruses do not pose a risk to animals due to their host specificity. An assessment regarding the possibility of spreading zoonotic viruses in the animal population (e.g. for hepatitis E virus and rotavirus) and regarding animal pathogenic viruses, was not carried out here.

In the case of plants that are not consumed raw, adverse health effects from irrigation with reclaimed wastewater are not to be expected according to current knowledge, as long as it can be ensured that sufficient heating of the food takes place before consumption.

[1] Growing plants in aqueous nutrient solution without soil

The inclusion of indicator viruses in the testing scheme of Regulation (EU) 2020/741 for the validation of wastewater treatment processes seems reasonable. However, the regulations and parameters currently listed in Annex I, Section 2 of Regulation (EU) 2020/741 for the validation of treatment facilities are not considered sufficient to ensure the safe removal of human pathogenic viruses from wastewater. A guideline of the World Health Organization (WHO) shows that higher requirements are necessary to achieve the absence of human pathogenic viruses. In the Guidance on Potable Reuse (WHO, 2017), a reduction of 9.5 log_{10} levels of enteric viruses (noroviruses) is recommended as a default performance target for the safe treatment of wastewater for use as drinking water. However, in order to be able to evaluate the effectiveness of virus reduction for human pathogenic viruses, suitable detection methods are required, which may still have to be developed or validated. If surrogate viruses are to be used for this purpose, evidence would also have to be provided that the behaviour of the surrogate viruses used is comparable to that of the human pathogenic viruses. This also applies to coliphages as currently common indicator viruses.

The parameters listed in Annex I, Section 2 of Regulation (EU) 2020/741 on monitoring the quality of reclaimed wastewater do not allow any statement on the presence of human pathogenic viruses. Therefore, the inclusion of indicator viruses in this catalogue of tests would also make sense. For this purpose, the suitability of various indicator viruses has to be examined with regard to their informative value on the presence and absence of human pathogenic viruses.

3 Rationale

3.1 Risk assessment

3.1.1 Hazard identification

3.1.1.1 Norovirus

Norovirus (NoV) is a non-enveloped single-stranded RNA virus classified in the genus *Norovirus* of the virus family *Calicividirae.* There are several genogroups with numerous genotypes (Chhabra et al., 2019). The most important human pathogenic NoV strains are classified in genogroups I and II.

According to the current state of knowledge, human NoV strains only infect humans and are not transmitted zoonotically to animals. The viruses are ingested orally, replicate in the gastrointestinal tract and are excreted in the stool. Transmission can occur either through direct contact with infected persons or indirectly via contaminated surfaces or ingestion of contaminated water or food (van Beek and Koopmans, 2013). In the latter case, contaminated mussels and frozen berries play a special role, often leading to food-borne outbreaks.

Little is known about the stability of human NoV in the environment and under specific physicochemical conditions. This is mainly due to the fact that suitable methods for infectivity testing in human NoV are largely lacking. For example, there are currently no efficient cell culture systems or animal models for human NoV. A recently developed cell culture system based on so-called intestinal organoids - organ-like cell structures grown in the laboratory - is promising (Ettayebi et al., 2021), but very labour-intensive and can only be used for a few selected questions. Many experiments to estimate the stability of human NoV have therefore been carried out using closely related viruses (so-called surrogate viruses) that can be easily propagated in cell cultures, such as murine NoV, Tulane virus or feline calicivirus. Other studies use molecular biological methods that investigate the integrity of the human NoV capsid through its binding to receptor molecules or through its protection of the viral genome from RNA-degrading enzymes. However, the informative value of data generated by surrogate viruses or molecular biological methods for the behaviour of human NoV is generally limited, and comparative studies often show big differences between the different virus species and methods used (Richards, 2012).

Some direct findings on the course of infection with human NoV and its inactivation were obtained with the help of infection experiments with volunteers. These showed that human NoV is very stable under various physico-chemical conditions. For example, treatment with 10 mg/l chlorine (Keswick et al., 1985) or heating for 30 min at 60 °C (Dolin et al., 1972) did not completely inactivate the virus, and high-pressure treatments of 600 MPa for five minutes were necessary to completely inactivate human NoV in oysters (Leon et al., 2011). However, in a recent study using intestinal organoid cell culture to measure the infectivity of human NoV, virus inactivation was observed after heating to 60 °C for 15 min (Ettayebi et al., 2016).

3.1.1.2 Hepatitis A virus

Hepatitis A virus (HAV) is classified in the genus *Hepatovirus* of the virus family *Picornaviridae*. It has an unenveloped viral capsid and a genome consisting of single-stranded RNA. Human HAV strains are classified as genotypes I-III (Smith and Simmons, 2018).

Representatives of genotypes I-III have so far only been detected in humans; closely related HAV strains of genotypes IV-VI are common in various monkey species. HAV is ingested orally and then replicates mainly in the liver. It is excreted in the stool. Virus transmission can occur through direct contact with infected individuals or via contaminated water and food (Bosch and Pinto, 2013). Above all, mussels and frozen berries can be contaminated with HAV.

Several data from different studies are available on the viral stability of HAV to environmental influences. However, because HAV field strains are difficult to isolate in cell culture, these data are based exclusively on a few cell culture-adapted strains, especially strain HM-175. It is therefore often difficult to interpret the data in terms of circulating HAV field strains. Based on the cell culture studies, HAV is among the most stable viruses tested to date against various physicochemical conditions. In mineral water, infectious HAV was still present after 300 days at room temperature (Biziagos et al., 1988). In an aqueous solution with added protein, sucrose and glycerol, infectious HAV was still detectable after 10 h of heat treatment at 60 °C, but with a titer decline of about five $\log_{10}$ levels (Murphy et al., 1993). HAV also shows high acid stability; infectious virus was still detectable after five hours at pH 1 (Scholz et al., 1989).

3.1.1.3 Other enteric viruses

Various other human pathogenic viruses can be transmitted via the faecal-oral route as well as via contaminated water and contaminated food. These include the hepatitis E virus and various gastroenteritis pathogens (Johne and Albert, 2013). For these viruses, however, there is little data on the significance of faecally contaminated plant foods as their vehicle. Other transmission routes are considered more significant.

Hepatitis E virus (HEV) is a non-enveloped single-stranded RNA virus classified in the family *Hepeviridae*. The most important human pathogenic virus strains are divided into genotypes 1 - 4. While genotypes 1 and 2 exclusively infect humans, genotypes 3 and 4 are zoonotic and have an animal reservoir in domestic and wild pigs. Genotypes 1 and 2 occur mainly in developing countries in Asia and Africa, where they are transmitted primarily through contaminated drinking water. In contrast, genotypes 3 and 4 are predominantly found in industrialised countries, where they are mainly transmitted via food produced from infected animals (Johne and Albert, 2013). In Germany, genotype 3 is the most common. Alternative transmission routes of this genotype, for example via faecally contaminated food (by humans or pigs), are suspected (Treagus et al., 2021). Little is known about the stability of HEV because of the lack of widely available efficient cell culture systems to measure infectivity. Based on a cell culture-adapted HEV genotype 3 strain, high stability to pH 2-9 was demonstrated, while the virus was inactivated at pH 1 and 10 (Wolff et al., 2020a). In solutions containing up to 20 % common salt with and without the addition of sodium nitrate or sodium nitrite, the virus was similarly stable as without the addition of these salts (Wolff et al., 2020b). In long-term experiments in phosphate-buffered saline, infectious virus was still detectable after 30 days at 22 °C (virus titer decline: 3.5 $\log_{10}$ levels) and after 60 days at 4 °C (virus titer decline: three $\log_{10}$ levels) (Johne et al., 2016).

Rotavirus is a non-enveloped RNA virus classified in the family *Reovidirae*, which can cause gastroenteritis, especially in young children. The most important species for human infections is rotavirus A, but it also occurs in animals. There are typical human pathogenic genotypes that are transmitted from person to person, but zoonotic transmissions from animals to humans can also occur. Rotaviruses are mainly transmitted through direct contact between children or via contaminated surfaces, but in individual cases transmission via contaminated food has also been demonstrated (Johne and Albert, 2013). The infectivity of rotavirus was unchanged after 15 days of incubation in groundwater and surface water at room temperature; in groundwater, infectious rotavirus was still detectable after several months and the infectivity decreased only about 10-fold after 120 min with the addition of 2 mg/l free chlorine (Espinosa et al., 2008). Infectious rotavirus was still detectable in water after heating at 50 °C for 15 min, but was completely inactivated under the same conditions at 55 °C (Ward et al., 1980).

Sapovirus is a gastroenteritis pathogen that, like NoV, belongs to the family *Caliciviridae*, but is classified here in the genus *Sapovirus.* The non-enveloped RNA virus is transmitted primarily from person to person via direct contact or contaminated food (Johne and Albert, 2013). Data on the stability of human sapovirus strains are not available due to the lack of cell culture systems. The significance of data from studies conducted with porcine sapovirus as a surrogate virus for the behavior of human sapovirus is unclear.

Astrovirus is a non-enveloped RNA virus classified in the family *Astroviridae*. The gastroenteritis pathogen is transmitted from person to person through direct contact. There is probably also indirect transmission via contaminated surfaces and contaminated water as well as food, although concrete evidence for this is still lacking (Johne and Albert, 2013). Probably due to the comparatively mild symptoms caused, there are only few studies on the stability of human astroviruses.

Adenovirus is classified in the family *Adenoviridae*. It is a non-enveloped DNA virus. Human adenoviruses are divided into numerous types that cause respiratory diseases, eye infections or gastroenteritis. Gastroenteritis pathogens are primarily adenovirus types 40 and 41, but other types can also lead to gastrointestinal symptoms (Johne and Albert, 2013). Adenoviruses are transmitted from person to person via direct contact as well as via contaminated water, probably also via contaminated food. In contrast to the other virus types mentioned above, various human adenovirus types can be easily isolated from stool and water samples using cell culture. Studies in wastewater showed little effect on the infectivity of adenovirus after 24 h incubation at 7 °C or 20 °C, while at 37 °C a decrease of more than five $\log_{10}$ levels was observed (Carratala et al., 2013). Another study showed that adenoviruses in deeper layers in sewage sludge can remain infectious for a very long time (T_{90} = 156 d) in winter months (Schwarz et al., 2019).

3.1.2 Hazard characterisation

3.1.2.1 Norovirus gastroenteritis

NoV usually causes gastroenteritis in humans, which is initially characterised by malaise followed by severe vomiting and/or diarrhoea. All age groups are affected. Symptoms usually last between 12 and 60 hours before clinical improvement and recovery occur (van Beek and Koopmans, 2013). In Germany, a total of 78,706 cases were reported to the Robert Koch Institute (RKI) in 2019 and a total of 28,532 cases of NoV gastroenteritis in 2020 (RKI 2021a). It is assumed that this decrease is due to hygiene guidelines to contain the COVID-19 pandemic. There are no vaccines against NoV infections yet.

After infection of volunteers, human NoV was excreted on average in concentrations up to 95 x 10^9 genome copies/g stool, with some of the volunteers showing maximum excretion levels between 10^{11} and 10^{12} genome copies/g stool (Atmar et al., 2008). Human NoV has a very high infectivity: outbreak studies determined a minimum infectious dose of 10 - 100 virus particles (Todd et al., 2008); an evaluation of studies on volunteers determined an infection probability of 50% with an ingestion of one NoV particle, although the triggering of disease only occurred at higher virus doses (Teunis et al., 2008).

In the past, numerous NoV gastroenteritis outbreaks have been described in connection with the consumption of plant-based foods. The largest foodborne outbreak described in Germany to date, with almost 11,000 cases, occurred in 2012 (Mäde et al., 2013; Bernard et al., 2014). It was triggered by NoV-contaminated frozen strawberries imported from China. Faecal contamination of the berries during cultivation or harvesting was suspected as the cause (Mäde et al., 2013). A later analysis showed that only very small amounts of virus (on average only about 200 NoV genome copies/25g) were detectable on the frozen strawberries, but these were apparently sufficient to trigger the large outbreak (Bartsch et al., 2018). A systematic review of 152 viral outbreaks worldwide following the consumption of fresh produce identified NoV as the causative agent (48.7%) and frozen raspberries (23.7%) as the most common vehicle of transmission (Chatziprodromidou et al., 2018). Another retrospective analysis of 606 disease outbreaks in the USA caused by consumption of leafy vegetables identified NoV as the cause in 55% of confirmed outbreaks (Herman et al., 2015).

3.1.2.2 Hepatitis A

Infections with **HAV** can lead to acute liver inflammation. The symptoms of the disease are initially flu-like and/or gastrointestinal symptoms characterised by skin itching, upper abdominal pain, jaundice and/or elevation of liver enzyme levels in the blood (RKI, 2021b). Severe courses of the disease occurred in older people and patients with previous liver damage. The global case fatality rate is reported to be 0.5 % (WHO, 2021). The recovery phase usually lasts between two and four weeks (RKI, 2021b). In Germany, a total of 873 hepatitis A cases were reported to the RKI in 2019 and a total of 561 hepatitis A cases in 2020 (RKI, 2021a). Effective vaccines against hepatitis A are available and are recommended by the Standing Commission on Vaccination (STIKO), especially for travel to regions with high hepatitis A prevalence.

Excretion of HAV by patients occurs in the stool for several weeks. In a study on 27 patients in the Netherlands, a mean excretion time of 81 days and concentrations of 2 x 10^6 to 2 x 10^8 genome copies/g stool were measured on day 36 after infection (Tjon et al., 2006). The estimated minimum infective dose is given as 10-100 particles (Todd et al., 2008).

Hepatitis A outbreaks associated with the consumption of plant-based foods have been frequently described in the past. Between January 2013 and August 2014, a hepatitis A outbreak occurred in 13 EU countries (including Germany), with 1,589 patients, of whom 1,102 were hospitalised (Severi et al., 2015). Consumption of food prepared with a frozen berry mix was identified as the cause. Other hepatitis A outbreaks have been described, for example, from HAV-contaminated dried tomatoes with 59 ill persons in France (Gallot et al., 2011), or from HAV-contaminated spring onions with 601 ill persons in the USA (Wheeler et al., 2005). A systematic review of 152 viral outbreaks worldwide following the consumption of fresh produce identified HAV as the causative agent in 46.1% of the outbreaks (Chatziprodromidou et al., 2018).

3.1.2.3 Diseases caused by other enteric viruses

Infection with **HEV** can lead to acute hepatitis with symptoms similar to those described for hepatitis A. In addition, chronic HEV infections can occur in immunosuppressed transplant patients, leading to fatal liver cirrhosis. The worldwide case/fatality rate in young adults is reported to be 3 % (Pallerla et al., 2020). In Germany, 3,728 cases have been notified to the RKI in 2019 and 3,253 cases in 2020 (RKI, 2021a). No approved vaccines against hepatitis E are available in Europe. HEV can be shed by chronically infected patients at concentrations up to 1 × 10^{12} genome copies/g stool (Wang et al., 2018); the infective dose is unknown. No data are available on disease outbreaks via plant foods. A case-control study identified raw vegetables (among other factors) as a risk factor for the development of acute hepatitis E in Germany (Faber et al., 2018).

Rotavirus infections lead to severe gastroenteritis, especially in young children, which can be life-threatening without treatment. However, with sufficient oral or parenteral fluid intake, the infections are self-limiting and usually lead to recovery after a few days (Johne and Albert, 2013). 36,871 cases were reported to the RKI in 2019 and 6,478 cases in 2020 (RKI, 2021a). Efficient vaccines against rotaviruses are available and are generally recommended by the STIKO for young children. Reports of disease outbreaks caused by the consumption of plant foods are rare; in a gastroenteritis outbreak on a ship, in which rotavirus was identified as a causative (aetiological) agent along with other viruses, lettuce was identified as the outbreak vehicle (Gallimore, 2005).

Infections with **sapovirus** can lead to gastroenteritis in humans (Johne and Albert, 2013). There is currently little data on the number of cases in Germany and vaccines against sapoviruses are currently not available. There are no reports of outbreaks caused by plant foods, but several outbreaks have been described in connection with the consumption of other foods (e.g. mussels) (Oka et al., 2015).

Astrovirus is an enteric pathogen that can lead to gastroenteritis in humans (Johne and Albert, 2013). The number of cases in Germany is not known and there are currently no vaccines against astroviruses. Because people suffering from diarrhoea are rarely tested for astrovirus, only a few disease outbreaks caused by this virus have been described, and the exact sources of infection have usually not been identified (Mattison, 2021).

Different human **adenovirus** types can cause different diseases in humans. Among them are some types that are excreted faecally and ingested orally and can lead to gastroenteritis (Johne and Albert, 2013). Exact disease figures are currently not available for Germany and vaccines do not exist at present. Even though adenoviruses are regularly detected in some of the gastroenteritis patients examined, there is little information on disease outbreaks and their causative transmission vehicles (Mattison, 2021).

3.1.3 Exposure estimation and assessment

In the following sections on exposure, the focus is on human NoV and HAV, since these viruses have a very high relevance for food safety.

3.1.3.1 Concentration in wastewater and reduction by wastewater treatment

Data on the concentration in wastewater and reduction through wastewater treatment were made available to the BfR by the German Environment Agency (UBA) in the form of three publications. According to these, there are on average 10^5 - 10^8 NoV genome copies/l and 10^2 - 10^4 HAV genome copies/l in untreated wastewater (Cornel et al., 2018; Selinka et al. 2020). Conventional biological wastewater treatment is assumed to reduce the virus concentration by 80 - 99.9 % (Seis et al., 2016; Selinka et al. 2020). In the "worst case" scenario (80 % reduction), the concentration after conventional wastewater treatment would correspond to 2 $x10^7$ NoV genome copies/l and 2 $x10^3$ HAV genome copies/l. No data on the amounts of infectious human NoV and HAV are included in the studies. Further cleaning and disinfection measures beyond conventional wastewater treatment can additionally reduce the amount of virus. The WHO Guidance on Potable Reuse recommends a 9.5 $\log_{10}$ reduction of enteric viruses (NoV) as a default performance target for safe treatment of wastewater for use as drinking water (WHO, 2017). Even if this value does not represent a direct benchmark for concrete validation tests of wastewater treatment plants, it does indicate that a combination of several wastewater treatment processes is necessary to reduce the concentration of human pathogenic enteric viruses in the reclaimed wastewater to a level at which no adverse health effects are to be expected.

3.1.3.2 Stability of viruses in soil

Irrigation with inadequately treated wastewater can introduce human pathogenic viruses onto and into the soil. In general, the stability of viruses in soil depends on several factors, such as the composition of the soil, temperature and moisture, and varies between different viruses (Rao et al., 1986; Rezutka and Cook, 2004; Sanchez and Bosch, 2014). The BfR currently has no data on the stability of human NoV in soil. For murine NoV, a surrogate virus for human NoV, a one $\log_{10}$ reduction in infectivity was observed in soil after twelve days (Fallahi and Mattison, 2011).

HAV has been shown to remain infectious in soil for several weeks to months (Cook et al, 2018). In a study in which soil suspended in groundwater or was artificially contaminated, a 1-2 $\log_{10}$ reduction was determined after 12 weeks at 25 °C and of less than one $\log_{10}$ at 5 °C (Sobsey et al., 1986). In a similar study, no significant reduction in HAV infectivity was observed after 20 days at 10 °C and 23 °C (Blanc and Nasser, 1996).

3.1.3.3 Stability of viruses on plant foods

Irrigation with inadequately treated wastewater can transfer human pathogenic viruses to plant foods. The BfR currently has no data on the stability of human NoV on plant foods. It has been shown for various surrogate viruses that they can remain infectious for several days to weeks on different plant foods such as spinach, leaf lettuce and strawberries (Mattison et al., 2007; Hirneisen and Kniel, 2013; Esseili et al., 2015). Butot et al. (2008) have further shown that feline caliciviruses used as surrogate viruses for human NoV can maintain their infectivity on frozen berries and herbs for months.

The stability of HAV was investigated on various experimentally contaminated plant foods. The results of these studies show that the stability of HAV depends on various factors such as plant species and temperature. Overall, several studies indicate that HAV is relatively stable on plant foods and can remain infectious on plant foods for extended periods of time.

Stine et al. (2005), for example, investigated the stability of HAV on cantaloup melons, lettuce and peppers in a climate chamber in which various growth conditions were simulated. Over a period of 14 days, only a slight reduction in infectivity of 0.01-0.29 $\log_{10}$ values per day was observed under all growth conditions. In another study, a 1-5 $\log_{10}$ reduction in infectivity was measured on peppers after 14 days, depending on temperature and humidity (Lee et al., 2015). The virus was more stable at lower temperatures and higher humidity. At 25 °C and 50 % humidity, the reduction was two $\log_{10}$ values after 14 days. The stability of HAV on spring onions was also investigated at different temperatures (Sun et al., 2012). A one $\log_{10}$ reduction was achieved at 23.4 °C after more than five days, while at 3.1 °C it was more than three weeks. On spinach, a one $\log_{10}$ reduction was observed after four weeks at 5.4 °C (Shieh et al., 2009). On lettuce, Croci et al. (2002) found a two $\log_{10}$ reduction after nine days at 4 °C, while no infectious virus was detected on carrots after four days and on fennel after seven days at identical temperatures. In contrast, no significant reduction in infectivity was observed on blueberries after seven days of incubation at 4 °C or 21 °C (LeBlanc et al., 2019). The infectivity of HAV was also investigated on alfalfa seeds (Wang et al., 2013). No significant reduction was observed after 20 days. Within 50 days, a two $\log_{10}$ reduction was evident. Bidawid et al. (2001) investigated the stability of HAV on packaged lettuce. For this purpose, lettuce leaves were contaminated and packed under a protective atmosphere. After 12 days at 4 °C, the reduction was less than one $\log_{10}$ value, regardless of the type of packaging. A stronger reduction and bigger differences between different types of packaging were found at room temperature. The infectivity of HAV was also investigated on various berries and herbs frozen after contamination (Butot et al., 2008). The experiments showed that neither freezing nor storage for three months at -20 °C led to a significant reduction.

3.1.3.4 Uptake of viruses via the roots into plants

Urbanucci et al. (2009) were unable to detect human NoV RNA in either hydroponically or soil-grown leaf lettuce when the plants were irrigated with experimentally contaminated water. However, recent studies indicate that human NoV could be taken up by plants via the roots. For example, both human NoV RNA and protein were detected in the leaves of leaf lettuce and spinach after virus was directly transferred (inoculated) to the roots (Esseili et al., 2018). Human NoV RNA was also detected in hydroponically grown spring onions and romaine lettuce after irrigation with experimentally contaminated water (Dicaprio et al., 2012; Yang et al., 2018). However, the BfR currently has no data on the infectivity of human NoV taken up by plants via roots. For various surrogate viruses, infectious virus has been detected after uptake via roots into leaf lettuce, romaine lettuce, spinach, spring onions, kale, mustard and strawberries (Yang et al, 2018; Esseili et al, 2018; Dicaprio et al, 2012; Wang and Kniel, 2015; Wei et al, 2011; Hirneisen and Kniel, 2013). The uptake of infectious virus depended on various factors such as the cultivation of the plants in hydroponic systems or in soil, the amount of virus material introduced and the plant species.

In general, cultivation in hydroponic systems and the presence of high viral pathogen quantities (titres) seem to favour the uptake of infectious viruses. Calculations using a model that describes the uptake of human NoV via the plant roots based on various published data show that the probability of virus uptake is higher into lettuce grown in hydroponic culture than into lettuce grown in soil (Chandrasekraran and Jiang, 2018). However, the model also shows that in both cases, adverse health effects on humans from ingested viruses are possible (Chandrasekraran and Jiang, 2018). Differences between different surrogate viruses have also been found. For example, comparable amounts of viral RNA were detected in plant leaves for porcine sapovirus and Tulane virus, but only infectious Tulane virus was detected in the leaves (Esseili et al., 2018).

For HAV, viral RNA was detected in spinach and spring onions when grown in hydroponic systems with experimentally inoculated water (Hirneisen and Kniel, 2013). Furthermore, HAV-RNA was detected in onions after they had been grown in experimentally contaminated soil or hydroponic systems (Chancellor et al., 2006). However, the BfR currently has no data on the infectivity of HAV taken up via plant roots.

3.1.3.5 Occurrence of viruses in plant foods - examples

Various studies have shown that RNA from viruses relevant to food safety can be detected in plant foods. In a study of leaf lettuce sold in three European countries, HAV was detected in 1.32 % (4/304) of the samples, human NoV of genogroup I in 2 % (6/299) of the samples and human NoV of genogroup II in 2.95 % (8/271) of the samples (Kokkinos et al., 2012). In a UK study, 5.3% (30/568) of tested lettuce samples, 2.3% (7/310) of fresh raspberry samples and 3.6% (10/274) of frozen raspberry samples were positive for NoV (Cook et al., 2019), while in a French study, 15.5% (31/200) of frozen berry samples and 11.9% (25/210) of fresh lettuce samples were positive for human NoV (Loutreul et al., 2014). In an Italian study, HAV was detected in 1.9 % (18/911) of ready-to-eat vegetable samples (Terio et al., 2017). Some studies from non-European countries have also found higher prevalences of HAV in plant foods (Randazo and Sanchez, 2020). However, the studies mentioned do not contain any information on the infectivity of the detected viruses.

3.1.4 Risk characterisation

3.1.4.1 Possibilities and limits of risk characterization

A conclusive risk characterisation for the use of reclaimed wastewater for the irrigation of plants with regard to human pathogenic viruses is currently not possible. Above all, data on the reduction of the infectivity of the human pathogenic viruses of interest through wastewater treatment, on the stability of these viruses in the soil and on the plant, as well as on the uptake of these viruses by the roots into the plant and the virus infectivity after such uptake are largely lacking (see Chapter 3.1.3.). For human NoV, such data are mostly not available due to the lack of suitable laboratory methods to detect infectivity; for HAV, the available data are almost exclusively based on a single cell culture-adapted strain (see chapter 3.1.1). The available data for surrogate viruses often cannot be used directly to describe the behaviour of human viruses (Richards, 2012 and see Chapter 3.1.1.1). Since data from surrogate viruses (enteroviruses, rotaviruses and bacteriophages) were also predominantly used for risk assessment in an assessment by the World Health Organisation on the use of reclaimed wastewater for the irrigation of plants (WHO, 2006), the conclusions drawn therein can only be inadequately transferred to the most important human pathogenic viruses in this context (human norovirus and hepatitis A virus). Furthermore, available RNA-based data of the human viruses are not suitable for estimating the corresponding infectivity, which would be necessary for an assessment in most cases.

However, when considering the few available data for the human pathogenic viruses as well as the available data for the investigated surrogate viruses, it can be stated that in most cases the viruses show a very high stability towards various physico-chemical parameters (see chapter 3.1.1), in the soil and on the plant (see chapter 3.1.3.2 and 3.1.3.3). Uptake into the plant via the roots was shown in most studies, at least on the basis of viral RNA detection (see chapter 3.1.3.4). RNA of the viruses of interest has been detected on plant foods in several studies (see chapter 3.1.3.5) and a large number of viral disease outbreaks after consumption of plant foods have been documented (see chapter 3.1.2). Taken together, these study results show that transmission of human pathogenic viruses to humans via irrigation of plants with contaminated reclaimed wastewater and their subsequent consumption is possible and highly plausible. The diseases triggered are highly relevant to public health (see Chapter 3.1.2). As already explained in the first section of this chapter, an exact estimation of the transmission risks is not possible. However, most of the viruses of interest (especially human NoV and HAV) have a very low minimum infective dose (see Chapter 3.1.2), which means that even small amounts of virus can lead to illness after ingestion. On the other hand, high amounts of viruses are excreted in the stool, which suggests the possibility of high contamination of wastewater (see chapters 3.1.2 and 3.1.3.1). Based on the available data, the further risk characterisation for different irrigation systems is based on a "worst case" scenario, in which it is assumed that the virus infectivity is not sufficiently reduced by wastewater treatment and that the viruses are not sufficiently inactivated in the soil and on or in the plant (i.e. they can still cause diseases in humans).

3.1.4.2 Risk characterisation in the context of a worst-case scenario

3.1.4.2.1 Subsurface drip irrigation

It is possible that infectious human pathogenic viruses, such as NoV and HAV, get into the ground with the reclaimed wastewater and remain stable there for longer periods of time. Direct contamination of the plant parts growing above the ground with viruses present in the reclaimed wastewater seems very unlikely. However, it cannot be ruled out that infectious human pathogenic viruses reach above-ground plant parts through uptake via roots.

In this scenario, the probability of contamination of plant parts intended for consumption growing in the ground is to be assessed as high. The probability of contamination of plant parts intended for consumption growing above the ground is considered to be very low, although such contamination cannot be completely ruled out.

3.1.4.2.2 Drip irrigation

It is possible that infectious human pathogenic viruses, such as NoV and HAV, get into the ground itself as well as plant parts growing in the ground with the reclaimed wastewater and remain stable there for longer periods of time. Contamination of plant parts growing above the ground appears possible if plant parts close to the ground come into direct contact with reclaimed wastewater or if soil particles together with viruses get onto the plant. In addition, it cannot be ruled out that infectious human pathogenic viruses reach above-ground plant parts through uptake via roots.

In this scenario, the probability of contamination of plant parts intended for consumption growing in the ground is to be assessed as high. The probability of contamination of above-ground plant parts intended for consumption is assessed as low to moderate.

3.1.4.2.3 Furrow irrigation

It is possible that infectious human pathogenic viruses, such as NoV and HAV, get into the ground with the reclaimed wastewater and remain stable there for longer periods of time. Contamination of plant parts growing in the ground can be assumed. Contamination of plant parts growing above the ground with viruses seems possible if plant parts come into direct contact with the reclaimed wastewater, e.g. through water splashes from the ditches or overflowing ditches. In addition, it cannot be ruled out that infectious human pathogenic viruses reach plant parts growing above the ground through uptake via roots.

In this scenario, the probability of contamination of plant parts intended for consumption growing in the ground is to be assessed as high. The probability of contamination of above-ground plant parts intended for consumption is assessed as moderate to high.

3.1.4.2.4 Sprinkler systems

It is possible that infectious human pathogenic viruses, such as NoV and HAV, get into the ground with the reclaimed wastewater and remain stable there for longer periods of time. Accordingly, contamination of plant parts growing in and above the ground can be assumed.

In this scenario, the probability of contamination of plant parts intended for consumption growing in and above the ground is to be assessed as high.

3.1.4.2.5 Hydroponic culture

It is possible that infectious human pathogenic viruses, such as NoV and HAV, are present in the reclaimed wastewater and remain stable over longer periods of time. Contamination of plant parts growing in the water must be assumed. Contamination of other plant parts seems possible if they come into contact with the irrigation water. In addition, it cannot be ruled out that infectious human pathogenic viruses reach other plant parts through uptake via roots. Data from surrogate human NoV viruses indicate that the risk of infectious virus uptake is higher with hydroponic culture than with other irrigation systems.

In this scenario, the probability of contamination of plant parts growing in the water and intended for consumption must be considered high. The probability of contamination of other plant parts intended for consumption is considered low, although such contamination cannot be ruled out.

3.1.4.3 Assessment of the quality of the data

As already explained in more detail in the first section of chapter 3.1.4, the quality of the data currently available is low. This concerns the extensive lack of data on stability and inactivation of human NoV as well as the almost exclusive use of a single cell culture-adapted strain in the available studies on stability and inactivation of HAV. The comparability of the behaviour of surrogate viruses, for which numerous data are available, with that of human viruses is questionable (Richards, 2012) and these data can therefore only be used for evaluation to a limited extent. In the same way, RNA-based data and capsid stability data determined by molecular biology can only be used to a very limited extent for statements on the infectivity of these viruses, which is, however, decisive for the assessment of risks.

3.1.4.4 Need for further research

In order to generate data for further assessment of the health risks posed by human pathogenic viruses in the use of reclaimed wastewater, research would be needed in the following fields:

- Development of methods for measuring the infectivity of human pathogenic enteric viruses. This primarily concerns the development of cell culture-based methods for determining the infectivity of human NoV, which are currently only available to a very limited extent and are not very efficient. Cell culture methods would also have to be improved for HAV in order to be able to examine a larger variety of strains.

- Generate data on the stability and inactivation of human pathogenic enteric viruses against physico-chemical parameters, in soil and on the plant. This requires the prior development of infectivity assays. Alternatively, animal experiments or studies with volunteers can be examined, as far as permissible and ethically justifiable in individual cases.

- To generate data on the uptake and subsequent infectivity of human pathogenic enteric viruses via the roots into the plant. The same limitations to infectivity assays as described previously apply.

- Generate data on the inactivation of human pathogenic enteric viruses during the treatment of wastewater. The same limitations to infectivity assays as described previously apply.

- Development of methods to estimate the efficiency of inactivation of human pathogenic enteric viruses by different wastewater treatment regimes. For this purpose, the suitability of surrogate viruses could be investigated comparatively with human viruses.

- Development of methods to assess the quality of the reclaimed wastewater with regard to the absence of infectious human pathogenic enteric viruses. For this purpose, detection systems for the human viruses would have to be established, which ideally can distinguish between infectious and inactivated virus. Alternatively, corresponding detection systems could be developed for indicator viruses, whose suitability for statements on human viruses would have to be demonstrated beforehand.

3.2 Risk management options, recommended measures

3.2.1 Limiting the use of reclaimed wastewater for irrigation

Based on the results of the risk characterisation (Chapter 3.1.4), the BfR recommends not using reclaimed wastewater for the irrigation of plants, whose parts growing close to or in the ground are intended for raw consumption. This recommendation applies until appropriate treatment procedures and controls can ensure that no human pathogenic viruses are present in the irrigation water. According to the current state of knowledge, infectious human pathogenic viruses present in the reclaimed wastewater can get onto or into the edible parts of the plants using any of the irrigation systems and cause illness in humans after raw consumption.

When comparing the different irrigation systems, subsurface drip irrigation and hydroponic culture appear to be associated with the lowest probability of virus contamination of above-ground parts of the plant. However, there is a lack of data on the uptake of human pathogenic viruses via the roots into the plant and their subsequent infectivity in the above-ground part of the plant, although some data obtained on surrogate viruses suggest such an uptake. Therefore this type of irrigation with reclaimed wastewater cannot be recommended either. No data are currently available on viruses for the evaluation of irrigation of fruit trees and vineyards with reclaimed wastewater. However, there are also no indications of disease outbreaks caused by viruses after the consumption of fruits grown on trees or grapes. An efficient uptake of viruses via the roots of fruit trees and vines with subsequent transport over relatively long distances into the fruits seems rather unlikely according to the current state of science, which is why adverse health effects are not to be expected from irrigating these plants with reclaimed wastewater (minimum quality class A or B). However, this assessment is only valid if no irrigation takes place during which the fruits come into direct contact with the reclaimed wastewater.

In the case of plants that are not eaten raw, health risks from irrigation with reclaimed wastewater are not to be expected according to current knowledge as long as it can be ensured that the food is sufficiently heated before consumption.

For the irrigation of fodder crops, no concrete health risks arise for the viruses mainly considered here (human NoV and HAV), as these viruses do not pose a risk to animals due to their host specificity. An assessment regarding the possibility of spreading zoonotic viruses in the animal population (e.g. for HEV and rotavirus) as well as regarding animal pathogenic viruses was not carried out here.

3.2.2 Testing for viruses in the validation of wastewater treatment processes

The inclusion of indicator viruses in the testing scheme of Regulation (EU) 2020/741 for the validation of wastewater treatment processes appears to make sense, as the properties of viruses sometimes differ significantly from those of bacteria. The WHO guidance on potable reuse (WHO, 2017) recommends a 9.5 $\log_{10}$ reduction of enteric viruses (NoV) as a default performance target for safe treatment of wastewater for use as drinking water. Although this value does not represent a direct benchmark for concrete validation studies of wastewater treatment plants, it does indicate that a combination of several wastewater treatment processes is necessary to reduce the concentration of human pathogenic enteric viruses to a level at which no adverse health effects are to be expected. However, in order to evaluate the effectiveness of virus reduction for human pathogenic viruses, suitable detection systems are required, which may still have to be developed or validated. If surrogate viruses are to be used for this purpose, evidence would also have to be provided that the behaviour of the surrogate viruses used is comparable to that of human pathogenic viruses. This also applies to coliphages as currently used indicator viruses.

3.2.3 Virus testing for the monitoring of reclaimed wastewater

The parameters listed in Annex I, Section 2 of Regulation (EU) 2020/741 on monitoring the quality of reclaimed wastewater do not allow any statement on the presence of human pathogenic viruses. Therefore, the inclusion of indicator viruses in this catalogue of tests would also make sense. For this purpose, the suitability of various indicator viruses has to be examined with regard to their informative value on the presence and absence of human pathogenic viruses.

Further information on the BfR website on the topic:

BfR Opinion No 021/2020 "Reclaimed waste water: preventing bacterial pathogens on fresh fruit and vegetables":

https://www.bfr.bund.de/cm/349/reclaimed-waste-water-preventing-bacterial-pathogens-on-fresh-fruit-and-vegetables.pdf

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