Bundesinstitut für Risikobewertung

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Reclaimed waste water: preventing bacterial pathogens on fresh fruit and vegetables

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As a result of climatic changes affecting Germany and Europe, an increase in the use of reclaimed waste water for irrigation of plants is to be expected, including ready-to-eat crops. Appropriate requirements for this irrigation water are therefore being drawn up at an EU level. Against this background, the German Federal Institute for Risk Assessment (BfR) has worked with the Julius Kühn Institute (JKI) and the Max Rubner Institute (MRI) to analyse recent research findings on the occurrence of certain bacterial pathogens in reclaimed waste water as well as fruit and vegetables. The healthy properties of fruit and vegetables mean that most people—and even individuals particularly sensitive to foodborne infections—eat them either raw or only after minimal food preparation.

The most important bacterial pathogens that occur in waste water, and which could be consumed by humans by eating fruit or vegetables, are *Salmonella*, Shiga-toxin producing *Escherichia coli* (STEC) and *Listeria monocytogenes*. STEC are pathogenic *E. coli* strains, which can produce Shiga toxin that affects the human gut. When these bacteria cause illness in humans, they are also known as enterohaemorrhagic *E. coli* (EHEC). *Listeria monocytogenes* can lead to serious illness in pregnant women and people with a weak immune system.

Despite a comparatively low rate of detection on plant-based foods, sizeable outbreaks of foodborne illnesses occur regularly as a result of fruit and vegetables contaminated with human pathogens. Since food supply chains are frequently very long and because the fresh plant-based foods spoil comparatively rapidly, fruit and vegetables are often consumed well before potential outbreaks are identified and suspect foodstuffs are examined.

The risk of the general population contracting a salmonellosis or STEC infection following the consumption of ready-to-eat fruit or vegetables has so far been considered to be low in Germany. For pregnant women and people with a weak immune system in Germany, the risk of suffering from listeriosis after consuming ready-to-eat fruit and vegetables also continues to be considered low, despite the severity of the illness. If the plants grown to produce fruit or vegetables are irrigated with reclaimed waste water and then eaten raw (whether in whole or in part), however, these risks could increase.

To protect against foodborne infections, consumers are recommended to wash fresh fruit and vegetables thoroughly with drinking water before eating, to reduce the concentration of microbes present on the fruit/vegetable skin. Simply washing fruit and vegetables cannot remove all of the pathogens that may be present, however. Consumers are therefore advised to peel or blanch vegetables that grow near the soil to further reduce any risk of infection.

Pregnant women and individuals with weak immune systems (due to advanced age, pre-existing conditions or taking certain kinds of medicines) are advised to heat sprouts thoroughly before consumption. These two groups of people are further advised not to consume pre-packaged ready-to-eat salads. Instead, salads should be prepared just before eating from fresh ingredients washed thoroughly in drinking water.

BfR risk profile: Illness caused by Salmonella, STEC or *Listeria monocytogenes* on consumption of raw fruit or vegetables. Opinion no. 021/2020					
A Affected persons	**General population**				
B Likelihood of impairment to health from consumption of ready-to-eat fruit or vegetables [1]	Practically impossible	**Unlikely**	Possible	Probable	Certain
C Severity of impairment to health from consumption of ready-to-eat fruit or vegetables [2]	**No impairment**	**Mild impairment [reversible/irreversible]**	**Moderate impairment [reversible/irreversible]**	**Severe impairment [reversible/irreversible]**	
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 contradictory**		
E Controllability by the consumer [3]	Controls not needed	**Controllable with precautionary measures**	**Controllable by avoidance**	**Not controllable**	

BfR risk profile: Illness caused by *Salmonella*, STEC or *Listeria monocytogenes* on consumption of raw fruit or vegetables Opinion no. 021/2020					
A Affected persons	**Pregnant women, senior citizens, people with chronic conditions, young children**				
B Likelihood of impairment to health from consumption of ready-to-eat fruit or vegetables [1]	Practically impossible	Unlikely	**Possible**	Probable	Certain
C Severity of impairment to health from consumption of ready-to-eat fruit or vegetables [2]	**No impairment**	**Mild impairment [reversible/irreversible]**	**Moderate impairment [reversible/irreversible]**	**Severe impairment [reversible/irreversible]**	
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 contradictory**		
E Controllability by the consumer [3]	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 be read only in conjunction with the Opinion.

[1] Row B – Probability of an impairment to health
The likelyhood of an impairment to health depends above all on the species and quantity of pathogens ingested as well as individual factors affecting the consumer, such as the condition of their immune system.

[2] Row C – Severity of the impairment to health:
The severity of the impairment can vary depending on the species and quantity of potential pathogens ingested. Severe impairments to health are possible in particular during pregnancy as well as for infants and young children aged five or under, senior citizens, and persons with a weak immune system.

[3] Row E – Controllability by the consumer
Recommendations for action are given at the end of the Opinion. A summary of these is also presented in the last paragraph in the grey box on the previous page.

1 Subject of the assessment

The German Federal Institute for Risk Assessment (BfR), the Julius Kühn Institute (JKI) and the Max Rubner Institute (MRI) have prepared a joint assessment of the risks to human health from infectious pathogens following the consumption of raw fruit and vegetables irrigated by reclaimed waste water. The viability of human pathogens in plants is one topic addressed by the assessment. The background to this work is a European Commission Proposal for a Regulation of the European Parliament and of the Council on minimum requirements for water reuse (version dated 17 June 2019 and new recent research findings requiring assessment).

The present Proposal for a Regulation seeks to define minimum requirements for reclaimed urban waste water that is utilised for agricultural irrigation. Urban waste water is defined by Directive 91/271/EEC as domestic waste water or the mixture of domestic waste water with industrial waste water and/or run-off rain water. Reclaimed urban waste water is therefore the resultant outflow from a waste water treatment facility in which domestic waste water or mixed waste water is treated.

On the strength of the proposed Regulation, EU member states can enact legislation to permit the irrigation of plants with reclaimed urban waste water. Alternatively, Member States may prohibit (whether in whole or in part) the use of reclaimed urban waste water in this way on their territories.

Before such a usage is permitted, a risk assessment must be performed that considers environmental risks as well as risks to human and animal health. The risk assessment to be used as the basis for the usage decision about reclaimed urban waste water must take into account not only the EU Food Hygiene Regulation but also the existing legislation governing surface, ground and drinking water (Directives 2000/60/EC, 2006/118, 2008/105/EC, 98/83/EC), contaminants (Regulation No 1881/2006) and machinery (Directive No 396/2005).

Depending on the results of the risk assessment, other quality requirements may also be set for the irrigation water. These requirements may, for example, cover heavy metals, pesticides, disinfectants, medicines, other substances of increasing concern or microorganisms with antimicrobial resistance.

The Proposal for Regulation dated 17 June 2019 includes details about the potential use of reclaimed waste water, requirements concerning its condition, further details about frequency of monitoring and requirements concerning the validation of the treatment method. To provide a convenient overview, Table 1 summarises the details from this proposal that are of importance for the quantitative detection of *Escherichia coli* as an indicator for the occurrence of gut bacteria.

The present Opinion addresses the potential direct transmission of *Salmonella*, Shiga-toxin producing *Escherichia coli* (STEC) and *Listeria monocytogenes* from reclaimed waste water, as well as indirect transmission from irrigated soil into plants. Furthermore, food of plant origin can be contaminated via these pathways with other human pathogenic bacteria or bacteria with antimicrobial resistance, viruses, parasites and chemical substances.

Table 1: Excerpt from the European Commission Proposal for a Regulation of the European Parliament and of the Council on minimum requirements for water reuse (version dated 17 June 2019).

Reclaimed water quality class	Indicative technology target	*E. coli* (CFU/100 ml) >90% of samples	*E. coli* (CFU/100 ml) <10% of samples	Minimum monitoring frequencies	Crop category	Irrigation method
A	Secondary treatment, filtration, and disinfection	≤10	≤100	Once a week	**All food crops, including root crops consumed raw** and **food crops** where the edible part is in direct contact with reclaimed water	All irrigation methods
B	Secondary treatment, and disinfection	≤100	≤1,000	Once a week	**Food crops consumed raw** where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops to feed milk- or meat-producing animals	All irrigation methods
C	Secondary treatment, and disinfection	≤1,000	≤10,000	Twice a month	**Food crops consumed raw** where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops to feed milk- or meat-producing animals	Drip irrigation or some other irrigation method that avoids direct contact with the edible part of the plant
D	Secondary treatment, and disinfection	≤10,000	≤100,000	Twice a month	Industrial and energy crops, and seeded crops	All irrigation methods

2 Results

Following an evaluation of the literature as well as internal research results, the BfR, the JKI and the MRI conclude that the occurrence of *Salmonella*, STEC and *Listeria monocytogenes* is also possible in reclaimed waste water. The probability is proportional to the concentration of faecal indicator bacteria (faecal coliform bacteria or *Escherichia coli*). The concentrations of pathogenic bacteria in irrigation water can further increase if the bacteria propagate in biofilms, which can form in the downstream piping and hose lines of the irrigation system as a result of the higher concentration of nutrients in the reclaimed waste water.

The probability of direct transmission of these pathogenic bacteria from the reclaimed waste water or indirectly via the irrigated soil onto or into fruit and vegetables intended to be eaten raw depends on numerous factors, including: the ambient conditions (e.g. temperature, humidity) and nutrients present; the characteristics and quantities of bacteria in the irrigation water and in the soil; the soil quality; the plant species; and any competing microorganisms in the soil and on the crops. The irrigation methods used to distribute the reclaimed waste water during crop cultivation are expected to be also an important factor. On the basis of the literature evaluated, it is estimated that the probability of transmission of human pathogens from the reclaimed waste water is lowest for below-ground drip irrigation systems, and highest for sprinkler systems and hydroponic cultivation[1].

It is possible for *Salmonella*, STEC and *Listeria monocytogenes* to survive on fresh fruit and vegetables, and then multiply on the plants or in the food prepared from these plants, if refrigeration is inadequate and if sufficient nutrients are available (e.g. through leaking plant juices). In rare cases, the pathogens can also invade the plant tissue.

While available data from the food control authorities of German federal states together with research data from the MRI do not permit a valid statement about the occurrence of *Salmonella*, STEC and *Listeria monocytogenes* on or in fresh fruit and vegetables, the data nonetheless indicate that these pathogens have so far been detected only rarely and in low quantities in these foods in Germany. In addition, only isolated foodborne outbreaks have been reported for these foods in Germany to date. Accordingly, the general population's risk of contracting a salmonellosis or EHEC infection following the consumption of these foods has so far been estimated as low.

Young children in Germany generally have a higher risk of contracting an EHEC infection. Since STEC rarely occur in fresh fruit and vegetables, the risk of falling ill following consumption of these foods has also been estimated as low for this group of individuals to date. This risk could increase, however, if STEC are transmitted onto soils and directly onto the edible parts of plants with the reclaimed waste water.

Listeriosis following the consumption of these foods while raw is possible only for pregnant women and people with a weak immune system. Despite the severity of the illness, this risk is also estimated as low as a result of the low prevalence of *Listeria monocytogenes* in these food groups in Germany. This risk could increase as a result of irrigation with reclaimed waste water, however.

In contrast to DIN 19650 ('Hygienic concerns of irrigation water'), the microbiological criteria given in Annex I of the present Proposal for a Regulation (dated 17 June 2019) (see Table 1)

[1] Cultivation of crops in water that has been enriched with nutrients.

do not stipulate any routine monitoring of the occurrence of *Salmonella* or enterococci (referred to in DIN 19650 as 'faecal streptococci'). The DIN also recommends further usage restrictions if the irrigation water contains more than 100 enterococci or 200 *E. coli* in 100 millilitre (ml). The microbiological criteria of the proposed Regulation (Table 1) are nonetheless comparable with the recommendations made in Commission Notice 2017/C 163/01 (Amtsblatt der Europäischen Union, 2017) and the requirements stipulated by ISO 16075-2:2015 (Beuth Verlag, 2015). However, they are considerably higher than the limit values recommended for faecal coliforms in a guideline published by the US Environmental Protection Agency (EPA) (EPA, 2004) on water reuse. In addition, the EPA also recommends daily microbiological monitoring of the reclaimed waste water.

The microbiological criteria specified in the current version of Regulation (EC) No 2073/2005 for the protection of consumers apply only to selected categories of food, and are not generally applicable to fresh fruit and vegetables. Accordingly, it is possible that contamination remains undetected, causing major salmonellosis or EHEC outbreaks, because fruit and vegetables can be marketed across very large regions, and are very frequently consumed raw by nearly the whole population. Consequently, it is especially important to prevent the introduction of human pathogens into the food chain via reclaimed waste water.

Accordingly, the BfR, the JKI and the MRI recommend the following to protect against foodborne illness caused by pathogenic bacteria, viruses and parasites resulting from the consumption of raw fresh fruit and vegetables:

1. For the hydroponic cultivation of food crops consumed raw, use only irrigation water of drinking water quality. This is because research findings indicate that pathogenic bacteria can easily colonise the roots and can then invade plants cultivated in this way via the roots. Pathogens must not be detectable in the irrigation water.
2. Restrict reclaimed water of quality category B to distribution using above-ground or below-ground drip irrigation. This is because these irrigation methods avoid direct contact with the edible part of the plant.
3. Restrict reclaimed waste water of quality category C to the irrigation of fruit trees, vineyards, feed plants and food products of plant origin that are not consumed raw.

To protect against foodborne infections, consumers are also recommended to wash fresh fruit and vegetables thoroughly with drinking water before eating, to reduce the concentration of microbes present on the fruit/vegetable skin. Simply washing fruit and vegetables is not guaranteed to remove all of the pathogens that may be present, however. Consumers are therefore advised to peel or blanch vegetables that grow near the soil to reduce any risk of infection.
In addition, individuals with weak immune systems due to pregnancy, advanced age, pre-existing conditions or taking certain kinds of medicines are advised to protect themselves from foodborne infections by heating sprouts thoroughly before consumption. These groups of people are further advised not to consume pre-packaged, ready-to-eat salads. Instead, salads should be prepared at home just before eating from fresh ingredients that have been thoroughly washed.

3 Rationale

3.1 Risk assessment

3.1.2 Hazard identification

3.1.1.1 Salmonella

Salmonella spp. are predominantly motile, Gram-negative, non-spore-forming rod-shaped bacteria and belong to the family *Enterobacteriaceae*. *Salmonella* are one of the most important bacterial zoonotic pathogens. Due to biochemical and serological investigation the genus *Salmonella* has two species, *Salmonella* (*S.*) *enterica* and *S. bongori*. *S. enterica* is further divided into six subspecies. *Salmonella* isolates can be categorised using the White-Kauffmann-Le Minor scheme on the basis of their somatic (O) and flagellar (H) antigens, and assigned by means of their seroformula to one of over 2,600 serovars (strains with identical antigen combinations).
Bacteria in the genus *Salmonella* are widespread in the environment and can be detected in many kinds of cold- and warm-blooded animals in all regions of the planet. *Salmonella* can be transmitted to humans via food. Most of the 2,600 serovars can occur in humans and in all animal species. Some serovars, such as *S*. Typhi, *S.* Paratyphi, *S*. Gallinarum and *S*. Dublin are host-specific, and typically found only in humans or in poultry and cattle. *Salmonella* can survive for several months in the environment and in - or on - various kinds of foods. *Salmonella* are tenacious and can even grow under extreme environmental conditions.

Compared with other bacteria, the growth requirements of *Salmonella* are low. *Salmonella* generally grow at temperatures ranging from 10 to 47 °C, and at a pH between about 4 and 9 (optimum pH is a value between 6.5 and 7.5). While some *Salmonella* strains grow at higher temperatures (up to 54 °C), others exhibit an increased tolerance to cold (psychotropic characteristics) and also grow in food stored at between 2 and 4 °C. The minimum a_w value[2] at which growth takes place lies between 0.92 and 0.95, depending on the substrate and temperature. In dry food, *Salmonella* can stay viable for a prolonged period of time at a_w values down to 0.43.

Freezing reduces the *Salmonella* colony count, but does not completely eliminate it. The viability of *Salmonella* during the storage of frozen food can be influenced by a number of factors. The composition of the matrix, the kinetics involved in the freezing process, the physiological condition of the *Salmonella* and serovar-specific properties all have a role to play. Studies describe the survival of *Salmonella* at −20 °C for 180 days on fruit and over a year in minced meat (Bardsley et al., 2019, Knudsen et al., 2001, Müller et al., 2012, Strawn and Danyluk, 2010).

At temperatures above 58 °C, *Salmonella* start to perish, although the heat resistance can be influenced by many different factors. In particular, heat resistance increases as water activity (a_w value[2]) decreases. Drying food at higher temperatures (50 °C and 61 °C) or storage at an elevated temperature and humidity (45 °C and 76 percent (%) relative humidity) also leads to a reduction of the *Salmonella* colony count. The extent of elimination of the bacteria is depending on the factors described above.

[2] a_w value: activity of water; parameter describing the availability of water in foods and/or prepared meals. The amount of water available for bacterial growth/metabolism is proportional to the a_w value.

When attempting to predict the success of the thermal inactivation of *Salmonella* in food, factors such as temperature, a_w value[2], and the fat or protein content of the food must concurrently be accounted for (Jin et al., 2018, Keller et al., 2018). Common salt has a bacteriostatic effect, primarily by binding available free water and therefore reducing the water activity. While growth rates slowly decline as common salt concentrations rise, the complete inactivation of *Salmonella* cannot be achieved merely by salting food.

3.1.1.2 STEC

Escherichia (E.) coli is a Gram-negative, non-spore-forming, rod-shaped bacterium that also belongs to the *Enterobacteriaceae* family. *E. coli* occurs naturally in the gut of animals and humans, and is therefore considered to be the most important microbial indicator for faecal contamination. Detection of *E. coli* in foods is a sign of inadequate hygiene during processing, operations or distribution. Certain strains of *E. coli* may trigger severe disease in animals and humans. Regarding illness in humans, *E. coli* strains able to produce Shiga toxins (Stx) (synonym: verotoxins) are of particular significance. Shiga toxin-producing *E. coli* (STEC) occur naturally in the gut of ruminants such as cattle, sheep and goats, as well as wild ruminants. Animals that excrete STEC do not display signs of illness. Bacteria enter the environment as well as various foods of animal and plant origin via the faeces. Moreover, direct transmission is possible between animals and humans, and from person to person. STEC that cause sickness in humans are described as enterohaemorrhagic *E. coli* (EHEC).

STEC are detected most frequently in the meat of wild ruminants during official food control (Hartung et al., 2016). However, STEC have also been detected in plant-based foods and foods with a low a_w value[2], such as flour or hazelnuts (Made et al., 2017, Miller et al., 2012). A study with artificially contaminated walnut kernels demonstrated the viability of *E. coli* O157 on this matrix for several months (Blessington et al., 2012).

STEC can grow at temperatures ranging from 8 to 45 °C. Since STEC are resistant to drying and freezing, they are able to survive weeks and months in the environment (soil, water, faecal matter). The decontamination of foods that are contaminated with *E. coli* O157:H7 using 0.5%, 1.0% and 1.5% organic acids has proven to be ineffective, and emphasise this pathogen's tolerance of acids (Brackett et al., 1994). STEC may also be insensitive to salt (Dupree et al., 2019). STEC start to die off at temperatures above 60 °C. D-values[3] for *E.coli* O157:H7 are known for foods such as meat and milk. Similar to other *E. coli* types, these D-values lie within a temperature range of 57–64 °C for heating times between 270 and 9.6 seconds. The fat content and drying of foods can also increase the D-value, however. One study on the viability of STEC of the serogroups O26, O103, O111 and O157 in wheat flour at temperatures of 55, 60, 65 and 70 °C showed that all strains tested survived for up to 60 minutes (min) (end of experiment) at 70 °C (Forghani et al., 2018). In the lab, a high level of viability following drying and storage on paper has also been demonstrated, with *E. coli* O157:H7 remaining viable at 70 °C for five hours (Hiramatsu et al., 2005).

The concentration of STEC in foods can also be reduced with the help of sulphur dioxide (SO_2), which is authorised as a preservative and anti-oxidation agent for various foods (E220) with product-specific maximum levels. Apart from its common use for dried fruits, SO_2 is, for example, also used in dried potato products and dried or frozen white vegetable varieties in which product-specific maximum quantities are permitted. A reduction in microbial

[3] The D-value is the time required for the reduction of a microorganism population to 10% at a given temperature.

populations of *E. coli* O157:H7 by up to five log levels, for example, was achieved in various sour apple juice products by using 50 ppm SO_2 (Basaran-Akgul et al., 2009).

3.1.1.3 Listeria monocytogenes

Listeriae are Gram-positive, rod-shaped, facultative anaerobic, non-spore-forming bacteria in the family *Listeriaceae*. Listeriae are actively motile by means of peritrichous flagella[4] at 20−25 °C. Because of their low growth requirements listeriae are widespread in the environment, and they occur in the soil, surface waters, waste waters, on plant remains and the gut of animals, for example (Ivanek et al., 2006, Lyautey et al., 2007, Paillard et al., 2005, Vivant et al., 2013). Effective adaptation mechanisms provide listeriae with a high tolerance against drying out (Vogel et al., 2010) and freezing, allowing them to survive in the environment (soil, water, faecal matter) at any time of the year (Strawn et al., 2013).
On account of its widespread distribution in the environment, *Listeria monocytogenes* can occur in a wide variety of foods. The highest detection rates are to be found in minced meat, raw meat preparations, smoked fish, cheese and delicatessen salads. However, many other kinds of foodstuffs can also be contaminated with *Listeria monocytogenes*, including particularly ready-to-eat foods that have not been subjected to any bactericidal treatment following their processing (EFSA and ECDC, 2017, Hartung et al., 2019).

Optimal growth conditions for *Listeria monocytogenes* are temperatures of 30 to 37 °C and a neutral to slightly alkaline pH. The pathogen is capable of propagating at temperatures between −1.5 °C and 45 °C, and pH values from 4.5 to 9. The pathogen's doubling time is strongly dependent on temperature, the a_w value[2] and the pH (Azizoglu et al., 2009, Hill et al., 1995, Liu et al., 2005, Wagner and McLauchlin, 2008). *Listeria monocytogenes* is relatively resistant to osmotic stress, and remains capable of growth even at high salt concentrations of up to 10% and a_w values of more than 0.92 (Nolan et al., 1992, Wagner and McLauchlin, 2008). Depending on the type of acid, the storage temperature and potential previous adaptation to low pH, *Listeria monocytogenes* can survive a decrease in pH to values as low as 3.0 (Liu et al., 2005). The pathogen remains viable at salt concentrations of up to 20% and can survive for long periods of time in dried foods, depending on the storage temperature (Kenney and Beuchat, 2004, Kimber et al., 2012, Koseki et al., 2015, Ryser and Marth, 2007). Freezing food at −20 °C does not significantly reduce the pathogen's colony count (Ryser and Marth, 2007).

Some stress factors, such as disinfectants, toxic metal ions, a lack of nutrients or unfavourable temperatures can cause the transition of *Listeria monocytogenes* to a viable but non-culturable (VBNC) state (Besnard et al., 2000, Highmore et al., 2018, Li et al., 2014, Robben et al., 2018). In this VBNC state, the bacteria no longer grow but rather remains in stasis. If detection methods dependent on obtaining live cultures are now used, this can result in false negatives, because the bacteria are not dead but merely inactive. Depending on the stress factor that induced the state, VBNC pathogens may be avirulent (Cappelier et al., 2005, Lindbäck et al., 2010) or as virulent as their culturable form (Highmore et al., 2018). Furthermore, the bacteria can subsequently return to their culturable—and infectious—state under the right conditions (Li et al., 2014).

Listeria monocytogenes can be reliably killed by heating food to a core temperature of 70 °C for at least two minutes. The D[3] and z values[5] as indicators of heat inactivation vary depend-

[4] Having flagella around the entire surface.
[5] The z value indicates the increase in temperature required to lower the D value by a tenth.

ing on the food matrix. Salt content and water activity are key factors here, as well as variability between bacterial strains (Aryani et al., 2015, van Asselt and Zwietering, 2006). The average D value[3] for a wide variety of matrices (meat and dairy produce were investigated in particular) at 70 °C is 0.09 min, with an average z value of 7.0 °C (van Asselt and Zwietering, 2006).

Various processes used in food technology are appropriate for reducing *Listeria monocytogenes* in or on the food. These include high-pressure treatment (Balamurugan et al., 2018, Garriga et al., 2004), the deployment of bacteriophages (Moye et al., 2018) or combinations of active antimicrobial substances (Batpho et al., 2017), UVC disinfection (Montgomery and Banerjee, 2015) and exposure to ozone gas (Nicholas et al., 2013). No single method is appropriate for use on all foods to the same degree, however, and its efficacy in terms of reducing the pathogen will depend strongly on the properties of the food itself, as well as its packaging and storage conditions. No method is currently known to be suitable for eliminating *Listeria monocytogenes* completely; instead, the pathogen can merely be reduced to a lower level. Any bacterial cells not sensitive to or not killed by the method remain viable and can, if conditions are suitable, continue to grow in ready-to-eat foods to levels exceeding the microbiological limits set by Regulation (EC) No 2073/2005.

3.1.2 Hazard characterisation

3.1.2.1 Salmonellosis

Salmonellosis is an infection caused by bacterial species in the *Salmonella* genus. The typhoid form (typhoid fever and similar diseases) is primarily caused by the serovars *S*. Typhi and *S*. Paratyphi A, B and C. Person-to-person transmission is possible. The pathogens are ingested orally and spread via the blood system. The infectious dose is low, and after a short incubation period[6] (a few days to three weeks) patients experience a severe, cyclic and systemic infection with diarrhoea and a high fever. Organ damage can occur in the gut, heart, liver, kidneys and gallbladder. In patients with gallstones, the pathogens can be shedover long periods of time.

In humans, most other *Salmonella* serovars cause the 'enteritic' form of the infection (enteritis = inflammation of the intestines). The infectious dose for adult humans is 10^4 to 10^6 *Salmonella* cells. If *Salmonella* is present in very fatty food such as cheese, hamburger, chocolate or salami or in case of special disposition of patients infections caused by lower than 100 cells of *Salmonella* has been observed. The adaptation of *Salmonella* to the unfavourable environment found on and in plants (such as adjustments to low pH) may mean that lower quantities of pathogens are sufficient to cause an infection in humans (Brandl, 2006).

The incubation period for infections with enteritic *Salmonella* is 5 to 72 hours (a maximum of seven days) and depends on the infectious dose. In humans, salmonellosis typically starts suddenly with severe watery diarrhoea (which may become bloody in the course of the infection), often accompanied by fever, nausea, vomiting and stomach aches or headaches. Symptoms typically last only a few hours or days. In severe clinical cases, chills, high fever, fainting and other systemic clinical symptoms will appear, with a typhoidal progression. A mild or symptomatic progression is common, which also depends on the quantity of bacteria ingested. Patients excrete enteritic *Salmonella* for an average of 3 to 6 weeks, and several months in the case of infants. Long-term excretion exceeding six months is relatively rare.

[6] For foodborne illnesses, the incubation period is defined as the time between the consumption of the contaminated food and the appearance of the first symptoms of the illness.

Cases of severe clinical progression are rare, as are extra-intestinal infections, which may include pericarditis, neurological disorders, reactive arthritis, spondylitis or osteomyelitis. Salmonellosis is rarely fatal. High-risk groups include persons whose immune system is not yet fully developed (children under five years old) and people whose immune system is weakened as a result of old age or pre-existing conditions, for example.

In Germany, salmonellosis is a notifiable disease. The number of reported cases has more than halved in the period 2009 to 2016, while a rise in case numbers was once again recorded in 2017. In 2018, the number of reported infections fell again by 5.2%, however of the 13,529 reported cases of salmonellosis are still higher than those in 2016. In 2018, salmonellosis was once again the second most common notifiable bacterial gastrointestinal disease in Germany. As in previous years, the highest age-specific incidence was in children aged less than five years, with maximum incidence in infants. Both sexes were affected virtually equally (RKI, 2019).

In 2018, 45% of cases - reported with details of a specific serovar - were caused by *S.* Enteritidis and 33% caused by *S.* Typhimurium followed by *S.* Infantis (2.7%), *S.* Derby (1.5%) and *S.* Kentucky (0.9%). Other serovars comprised 17% of all cases with specified serovars. In 2018, a total of 14 confirmed cases of death in connection with salmonellosis were reported to the RKI (in 2017: 20). These included eight males and six females between 42 and 92 years old (overall median: 75 years). Six cases of death were attributable to the serovar *S.* Enteritidis, three cases to *S.* Typhimurium and one case to *S.* Agona. Four cases of death were reported without specific details of serovars (RKI, 2019).

In accordance with Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003, three strong-evidence salmonellosis outbreaks were reported by EU by Member States for 2017 that were associated with the consumption of vegetables, juices and similar products, with one caused by herbs and spices and one by cereal products (EFSA and ECDC, 2018a). Strong evidence is existent, if the results of microbiological and/or epidemiological investigations have been able to determine a link with high degree of probability between the identified food and the illness diagnosed.

According to figures from the Robert Koch-Institute, 125 of the 274 outbreaks caused by *Salmonella* in Germany in 2018 were recorded explicitly as foodborne outbreaks. Of these, no outbreaks were reported to have been caused by vegetables/vegetable products, fruit/fruit products or cereals/cereal products (RKI, 2019).

According to a report from the European Food Safety Authority (EFSA), most outbreaks of salmonellosis in humans are associated with the consumption of eggs and egg products, bakery goods, and meat and meat products. In contrast, the consumption of vegetables and fruit juices (and products thereof) was responsible for only 1.1% of the reported strong-evidence foodborne outbreaks (EFSA and ECDC, 2017).

Table 2 provides an overview of foodborne salmonellosis outbreaks that have been associated with the consumption of fresh products of plant origin.

Table 2: Salmonellosis outbreaks caused by the consumption of fruit and vegetables in the USA and Europe

Country	Year(s)	Food	Number of cases	*Salmonella* enterica serotype (outbreaks)	Literature
USA	2010	Alfalfa sprouts (lucerne sprouts)	140	*Salmonella* I	Harvey et al. (2016)
USA	1990-2010	Tomatoes	1959 (multiple outbreaks)	*S.* Newport (6), *S.* Braenderup (2), *S.* Baildon (1), *S.* Enteritidis (1), *S.* Javiana (1), *S.* Montevideo (1), *S.* Thompson (1), *S.* Typhimurium (1)	Bennett et al. (2015)
USA	1998-2010	Various types of sprouts	2-256	*S.* Enteritidis (4), *S.* Typhimurium (3), *S.* Saintpaul (3), *S.* Mbandaka (3) *S.* Braenderup (2), *S.* Newport (2), *S.* Cuba (2), *S.* Bovismorbificans (1), *S.* Muenchen (1), *S.* Kottbus (1), *S.* Chester (1), *S.* Oranienburg (1), *S.* Montevideo (1)	Dechet et al. (2014)
USA	2011	Vine tomatoes	33	*Salmonella* spp.	Harvey et al. (2016)
USA	2014	Cucumbers	275	*S.* Newport	Angelo et al. (2015)
Europe					
Austria, Belgium, Denmark, Germany, Italy	2011	Datterino tomatoes	71	*S.* Strathcona	Müller et al. (2016)
Germany, Netherlands	2011	Mung bean sprouts	106	S. Newport	Bayer et al. (2014)
6 EU countries	2011-2012	Watermelons	63	S. Newport	Byrne et al. (2014)
Norway	2013	Ready-to-eat bagged mixed salads	26	*S.* Coeln	Vestrheim et al. (2016)
Switzerland	2013	Presumed to be sprouts	13	*S.* Szentes	Nuesch-Inderbinen et al. (2015)
Germany and Switzerland	2014	Sprouts	74	*S.* Bovismorbificans	Knoblauch et al. (2015)

3.1.2.2 EHEC infections

Illnesses caused by EHEC may account mild to severe diarrhoea in humans. Haemolytic-uremic syndrome (HUS) is a potentially serious consequence of an infection, especially in young children. HUS manifests itself in form of acute kidney failure and a potentially fatal outcome. The following details concerning the infectious dose, incubation period and excretion period are generally based on findings for the O157:H7 EHEC serotype. The infectious dose is very low, with bacterial counts below 100 colony-forming units (CFU) (Paton et al., 1996, Teunis et al., 2004, Tilden et al., 1996). The incubation period is typically around 2 to 10 days (average of 3 to 4 days). Patients remain infectious while EHEC bacteria are detectable in their stool. Information about bacterial excretion periods varies significantly, from a few days to several weeks or months (Bai et al., 2018, Matussek et al., 2016). A Swedish study reported an average excretion period of 17 days (Bai et al., 2018).

A total of 2020 cases of EHEC infection and 95 cases of HUS were reported to the RKI in Germany in 2017. As in previous years, children under the age of five were particularly affected (29% of EHEC cases and 48% of HUS cases). EHEC infections occurred more frequently in females than in males. In 2017, 15% of EHEC cases reported with serogroup data were caused by O91 strains, 13% by O103 strains and 11% by O157 strains. A few cases of fatal outcome were reported. Eight confirmed fatalities associated with HUS cases and two fatalities resulting from EHEC-related illness were reported to the RKI in 2017 (RKI, 2018).

A current meta-analysis states fruit and vegetables as the reason for approximately 30% of traceable EHEC outbreaks between 1998 and 2017 in Europe (Pires et al., 2019). Approximately 35% and 42% of traceable EHEC outbreaks have been attributed to fruit and vegetables in North/South America and the West Pacific, respectively. Accordingly, fruit and vegetables are significantly more important than dairy produce or meat from ruminants (Pires et al., 2019). Table 3 provides an overview of vegetable-borne EHEC outbreaks in Europe over the last five years.

Table 3: Selected STEC/EHEC outbreaks caused by the consumption of fruit and vegetables in Europe (selection from 2013).

Country	Year	Food	Number of cases	Serogroup/serotype	Literature
Finland	2016	Rocket	237	ONT:H11 (STEC) O111:H8 (EPEC)	Kinnula et al. (2018)
UK	2015	Mixed salad (contaminated with sheep faeces)	47	O157:H7	Mikhail et al. (2018)
UK	2014	Coleslaw	20	O157	Byrne et al. (2016)
UK	2014	Presumed to be raw vegetables/salad	102	O157	Sinclair et al. (2017)
UK	2013	Watercress	22	O157	Jenkins et al. (2015)
UK	2013	Watercress (contaminated with cattle faeces)	6	O157	Jenkins et al. (2015)

3.1.2.3 Listeriosis

Not all Listeria species cause illness. Of the 20 species described in the genus *Listeria*, only *Listeria monocytogenes* is a significant cause of infection in humans. Listeriosis is a bacterial infection usually transmitted by food that is contaminated with the bacterium *Listeria monocytogenes.* Infection in humans is also generally possible via direct contact with infected animals or other people. Infections in humans resulting from these routes have rarely been described, however.

While the number of cases is low compared with other foodborne infections, listeriosis may cause very severe illness in people in certain risk groups and may therefore even prove to be fatal. Pregnant women, people with weakened immune systems and elderly people have an increased risk of contracting listeriosis. Although pregnant women typically exhibit only flu-like symptoms, the vertical transmission of listeriosis to the unborn child often causes sepsis in the foetus, combined with multiple organ manifestations that may cause premature birth, miscarriage or stillbirth. Neonates with listeriosis have a higher risk of lethality due to multiple organ failure and/or insufficient lung maturity (Lamont et al., 2011). Excluding cases of listeriosis in pregnant women, the majority of other cases are associated with hospitalisation and severe clinical symptoms such as blood poisoning, brain inflammation and/or meningitis, and organ manifestations that may include endocarditis or septic inflammation of the joints. In healthy individuals who do not belong to one of the risk groups, an infection with *Listeria monocytogenes* can cause inflammation of the gastrointestinal tract plus a fever, with progression generally being mild and self-limiting (Maertens de Noordhout et al., 2014).

The incubation period for invasive listeriosis is typically 8 days, and varies depending to the clinical form between 1 and 67 days. While long incubation periods of 17 to 67 days (average 27.5 days) occur in pregnancy-associated listeriosis, significantly shorter incubation periods of 1 to 14 days (average 9 days) are found in cases involving the central nervous system and 1 to 12 days (average 2 days) in case of bacteraemia. Non-invasive listeriosis leads to symptoms of illness within 6 hours to 4 days (average 24 hours) (Goulet et al., 2013).

The exact infectious dose for listeriosis is not known. Currently there are no reliable data on the potential minimum infectious dose of the invasive form of listeriosis, which would permit an estimate of a dose-response relationship. The infectious dose is crucially dependent on the individual's immune status, the virulence of the *Listeria monocytogenes* strain and also on the food matrix. Among other things, the type of food will influence the pathogen's ability to survive passage through the stomach (Buchanan et al., 2017, Hoelzer et al., 2013).

In Germany, the number of listeriosis cases reported in 2018 declined for the first time after a multi-year trend of rising incidences. A total of 701 listeriosis cases meeting the reporting requirements were notified to the RKI (versus 769 cases in 2017) (RKI, 2019). These included 659 cases of non-pregnancy-associated invasive listeriosis, 20 cases of pregnancy-associated listeriosis and 18 cases of listeriosis in neonates (including 12 mother-child pairs). As in previous years, the incidence of non-pregnancy-associated invasive listeriosis was significantly higher in elderly patients. Males were affected significantly more often than females. A total of 32 deaths were reported with listeriosis being stated as the cause of death (29 cases of non-pregnancy-associated listeriosis, 3 cases of neonate listeriosis). The case fatality rate in 2018 was therefore 5% (RKI, 2019).

A number of listeriosis outbreaks were described, in which raw plant-based foods had been identified as the source of infection: these included vegetables such as salad greens, sprouts, coleslaw or celery as well as fruits such as stone fruits and melons. An overview is

provided in Table 4. Contamination of the plant-based foods in question was typically reported—or at least suspected—during processing. One important factor is that the pathogen can persist in the production environment. However, a search is rarely made for the entry routes into the food processing facilities. Accordingly, it is typically unclear whether the pathogen entered production via the raw plant product or via other input sources.

The largest listeriosis outbreak caused by plant-derived foods described to date, totalling 147 cases, occurred in the USA in 2011 and was traced to cantaloupe melons contaminated with *Listeria monocytogenes*. The outbreak strain was not detected on fresh melons and in environmental samples taken directly in the field, but only on cantaloupe melons already processed and in the production environment of the agricultural business that cultivated the melons. Alongside a vehicle that had been used to drive rotten melons to a cattle farm, other potential input sources included a slight contamination of harvested products and the persistence of the pathogen in the production facility (McCollum et al., 2013).

Another listeriosis outbreak with 35 cases in the USA, lasting from 2015 to 2016, was caused by caramel apples contaminated with *Listeria monocytogenes*. The outbreak strain was detected in the crop-producing company's fruit cleaning area (polishing and drying brushes, conveyor belt and wooden containers) (Angelo et al., 2017).

Between 2015 and 2018, 32 people contracted listeriosis in five EU Member States, probably as a result of consuming frozen sweetcorn and other frozen vegetable mixtures. The frozen vegetables were not always heated before consumption, but merely thawed and then used as ingredient in fresh salads, for example. The source of the infection was traced to a manufacturer of frozen vegetables in Hungary. Here too, it is presumed that the vegetables were contaminated during processing, although the source of contamination could not be identified in the manufacturing company's production environment (EFSA and ECDC, 2018b).

The cited examples of listeriosis outbreaks show that plant-based foods contaminated with *Listeria monocytogenes* constitute a health hazard for sensitive individuals who have an elevated risk of contracting listeriosis. This applies to the raw consumption of contaminated plant-based foods in particular. The pathogen can also be transmitted to other ready-to-eat foods by cross-contamination, for example when contaminated plant-based foods are stored unprotected in the refrigerator. Plant-based foods not intended to be eaten raw can also cause listeriosis, if this advice against raw consumption is ignored or if they are used as an ingredient in meals that are then inadequately heated before consumption.

Table 4: Listeriosis outbreaks caused by the consumption of fruit and vegetables in North America and Europe

Year	Food	Country	Cases of infection	Food contaminated by	Literature
1981	Coleslaw	Canada	41	Possibly fertiliser	Schlech et al. (1983)
2008	Sprouts	USA	20	Not reported/unknown	Cartwright et al. (2013)
2010	Celery, cut	USA	10	Production environment, only cut celery contaminated	Gaul et al. (2013)
2011	Cantaloupe melons	USA	147	Production environment	McCollum et al. (2013)
2013/14	Salad, washed and cut	Switzerland	32	Production environment (conveyor belt)	Tasara et al. (2015)
2014	Mung bean sprouts	USA	5	Production environment	CDC (Centers for Disease Control and Prevention) (2015)
2014	Stone fruits (peaches, nectarines, plums, pluots)	USA	2	Not reported/unknown	Jackson et al. (2015) Chen et al. (2017)
2014/15	Caramel apples	USA	35	Apples, production environment	Angelo et al. (2017)
2015/16	Leafy green salads packed	USA Canada	28 (19 USA, 9 Canada)	Production environment	Self et al. (2019)
2015-2018	Frozen vegetables (sweet corn, mixed vegetables)	Denmark Finland Austria Sweden UK	32	Production environment	EFSA and ECDC (2018b)
2017	Caramel apples	USA	3	Not reported/unknown	Marus et al. (2019)

3.1.3 Exposure

3.1.3.1 Input sources, occurrence, survival and propagation of *Salmonella*, STEC and *Listeria monocytogenes* in soil

The most likely sources of human pathogenic bacteria in agricultural production systems such as soil or hydroponic facilities are organic fertilisers (such as wet manure, digestates from biogas plants, sewage sludge, etc.) as well as irrigation water (Allende and Monaghan, 2015, Barak and Liang, 2008, Beuchat, 2002, Jacobsen and Bech, 2012, Li et al., 2015, Miles et al., 2009, Olaimat and Holley, 2012). Among agricultural livestock, pigs and poultry are important sources of *Salmonella* while cattle are important sources for STEC (EFSA, 2008, EFSA and ECDC, 2017). Human pathogens are introduced to the soil via the spreading of organic fertilisers from animal husbandry. Furthermore, irrigation water can be contaminated with human pathogens, especially in cases where the water is sourced from ponds or drainage ditches situated closely to areas treated with organic fertilisers (Jacobsen and Bech, 2012) or reclaimed waste water is used. .

Urban waste water may contain many different types of bacteria in varying concentrations, including bacteria that are pathogenic in humans. The species and quantities of the bacteria present depend on their frequency of occurrence in the excreta of the humans and animals

from which the waste water is sourced. Various treatment methods can be used to reduce the concentrations of bacteria in reclaimed waste water. These methods include physical separation (e.g. sedimentation and filtration) as well as inactivation (e.g. with disinfectants, UV light and ozone).

To enable the microbiological state of the waste water to be monitored, samples are usually tested for the occurrence of microbes known as 'indicator bacteria' (total coliforms, faecal coliforms, *E. coli*): these bacteria occur in the gut of humans and warm-blooded animals in high concentrations, and their quantities in waste water can indicate the success rate of disinfection (US EPA, 2004). Koivunen et al. (2003) appraised the performance of various kinds of waste water treatment plants in Finland and found that faecal coliforms are a good indicator for the occurrence of *Salmonella* in urban waste water.

However, low concentrations of faecal coliforms or *E. coli* in reclaimed waste water do not necessarily guarantee the absence of human pathogens. A microbiological study performed in Saudi Arabia on reclaimed waste water showed that low colony counts of pathogenic bacteria (*Salmonella*, Vibrio, Listeria) and nematode eggs could be detected even at low concentrations of faecal coliforms (240 CFU per 100 ml) and *E. coli* (60 CFU per 100 ml) (Balkhair, 2016).
A study performed in southern Italy showed that the microbiological condition of the waste water used for irrigation depends on the waste water treatment procedure. Following treatment with ultrafiltration, the concentration of *E. coli* was around 10 CFU per 100 ml, while waste water reclaimed using a different treatment method contained around 1,400 *E. coli* per 100 ml. *Salmonella* were not detected in the samples of reclaimed waste water (Lonigro et al., 2015).

While the entry of human pathogens into the soil via organic fertilisers or irrigation water can be reduced by monitoring procedures, other sources are very hard or impossible to control. These include faecal matter from wild animals, insects or contaminated soil particles, which can be carried to neighbouring fields on the wind (Kumar et al., 2017). In wild animals, for example, *Salmonella* serovars were found in the excreta of magpies, red foxes, hedgehogs and rooks, for example (Rubini et al., 2016).

The survival of *Salmonella*, STEC and *Listeria monocytogenes* in the soil and other environmental habitats is a precondition for their ability to colonise new host organisms—such as plants (Winfield and Groisman, 2003). As recent research has demonstrated, *Salmonella* (and probably other human pathogenic bacteria as well) can adapt to the environmental conditions found in soil or in plants (Fornefeld et al., 2017, Hruby et al., 2018, Jechalke et al., 2019, Pornsukarom and Thakur, 2016). How this adaptation proceeds in detail, however, is largely unknown, therefore considerable research is needed on this topic.

Findings from many studies have shown that human pathogens can establish themselves in the soil at low levels and remain there over a prolonged period of time (Barak and Liang, 2008, Brennan et al., 2014, Hruby et al., 2018, Jechalke et al., 2019). Viable STEC have even been re-isolated from artificially contaminated soils after more than 100 days (Ma et al., 2011). In laboratory studies, the survival of *Listeria monocytogenes* has been shown in samples of chalky, cool soils for up to 1,500 days (Picard-Bonnaud et al., 1989b). This survival in soil samples had nearly no negative effects on the virulence of the pathogen (McLaughlin et al., 2011, Picard-Bonnaud et al., 1989a).

The persistence of human pathogens in the soil itself depends on a wide range of abiotic and biotic factors. Important abiotic factors that promote the persistence of human pathogens in

agricultural soils include nutrient availability and soil moisture (Alden et al., 2001, Lesk et al., 2016). Experiments conducted on a farm in Israel revealed that the survival of faecal coliforms and coliphages was proportional to soil moisture content (Campos et al., 2000, Oron et al., 2001). As a result, above-ground irrigation in the top layers of soil leads to higher concentrations of faecal indicator bacteria, with these clustered around the drip point in drip irrigation (Hidri et al., 2013, Malkawi and Mohammad, 2003, Palese et al., 2009, Sacks and Bernstein, 2011).

In field trials in Spain, concentrations of faecal coliforms in the soil declined slowly following the last irrigation with reclaimed waste water by about two log levels (from 3.6×10^4 CFU per 100 ml to 1.4×10^2 CFU per 100 ml) within 25 days. Where the fields had been irrigated subsequently with drinking water, the concentrations of faecal coliforms fell only slightly within the first few days as a result of the moisture, and then remained stable at around 10^3 CFU/g (Manas et al., 2009). In contrast, laboratory studies by Kouznetsov et al. (2004) revealed that above-ground irrigation was able to reduce faecal coliforms to 7% of their initial value within 72 hours. These faecal microbes were reduced even faster and more thoroughly by below-ground irrigation. The greatest stability in the experiments was exhibited by somatic coliphages.

The persistence of human pathogens in soil can also be influenced by pH, temperature or solar radiation (Callahan et al., 2017, Santamaria and Toranzos, 2003, Semenov et al., 2011). Lonigro et al. (2015) presume that the intensive solar radiation during the summer months in southern Italy had led to a situation where, despite the use of reclaimed waste water with up to 1,400 *E. coli* per 100 ml, neither *E. coli* nor *Salmonella* could be detected following the harvest, whether in irrigated soil or in vegetable samples tested. In Morocco, the upper soil layers (0–5 cm) also held slightly lower levels of faecal coliforms in summer than in winter (5–30 versus 8–116 CFU/g, respectively), depending on the quality of the irrigation water applied aboveground. At deeper levels of the soil (>25 cm), no more faecal coliforms were detectable even after irrigation with waste water (El Hamouri et al., 1996).

To be able to estimate and assess the significance of human pathogens in agricultural soils, it is also important to take proper account of the influence of agricultural management practices, such as the spreading of organic fertilisers as well as crop rotation (Jechalke et al., 2019). Current research is therefore investigating the persistence of *Salmonella* in soil-plant ecosystems affected by wet manure spreading and the degree of their influence (for a systematic overview, see Ongeng et al. (2015)). Results from the BLE project *plantinfect* (Justus Liebig University Giessen, 2018) show that *S.* Typhimurium strain 14028s, *S.* Typhimurium strain LT2 and *S.* Senftenberg can survive for several weeks in soil.

The study also showed that the persistence of the *Salmonella* strains investigated was higher in loamy soils than in sandy soils. The joint inoculation of *Salmonella* with organic fertilisers increased their rate of survival (Jechalke et al., 2019). A possible explanation for the elevated persistence of *Salmonella* in loamy soils could be their generally higher concentrations of nitrogen and organic carbon, as well as the higher clay content of these soils (Rühlmann and Ruppel, 2005). In contrast, an earlier study showed that the colony count of culturable *S.* Typhimurium LT2 in soils treated with sewage sludge decreased more quickly than in untreated soils (Fornefeld et al., 2018). The authors explain their results by suggesting that the stress caused by the sewage sludge spreading possibly caused the *Salmonella* to switch into a VBNC state (viable but not culturable), meaning that they were no longer capable of being cultured.

Based on research findings to date, we may assume that the persistence of human pathogens in soil depends on the availability and composition of nutrients as well as the soil type. Potentially, therefore, the future use of fertilisers whose formulation includes high concentrations of organic substances and low concentrations of easily available nutrients could be used to lower the persistence of *Salmonella* in soil—as has already been shown for *E. coli* O157:H7 (Franz and van Bruggen, 2008).

Alongside these abiotic factors, biotic factors also influence the persistence of human pathogens in soil. Significant biotic factors include the specific genetic makeup of the human pathogens, the autochthonous microbiota and the plant-specific root exudates. The last two influence the microbial community in the soil, for example (Berg and Smalla, 2009, Jechalke et al., 2019, Locatelli et al., 2013, Schlaeppi et al., 2014), which in turn influences the persistence of human pathogens in this soil. The structure and function of the autochthonous microbial soil community is of decisive importance for the persistence of human pathogens in soil. As the variety and metabolic activity of the autochthonic soil microorganisms increases, the persistence in soil of various human pathogens such as *Listeria monocytogenes*, *E. coli* O157:H7 (Locatelli et al., 2013, van Elsas et al., 2012, Westphal et al., 2011) and *Salmonella* (Schierstaedt et al., 2019) decreases. Moynihan et al. (2015) also cite the importance of biotic factors for the persistence of human pathogens in soil. The persistence of *S.* Dublin in soils with varying soil types as well as methods of soil management tends to correlate rather more closely with differences in the microbial communities than with differences in the soil's physiochemical properties (Moynihan et al., 2015).

To date, it remains entirely unclear how biotic variety and biotic resistance (resilience) behaves against newly invading human pathogens. It appears to be the case that ecosystems with a higher level of biodiversity offer less accommodating niches and/or a greater chance of hosting natural antagonists and competitors (Chapin Iii et al., 2000, Li and Uyttendaele, 2018, Loreau and Hector, 2001, Mallon et al., 2015), which may suppress the human pathogens. Conversely, it has been shown that the persistence of *E. coli* O157:H7 in soil correlates strongly with a low level of microbial diversity and an abundance of available nutrients (van Elsas et al., 2012, Westphal et al., 2011). Improved rates of survival in soils with less microbial diversity have also been observed for *Salmonella* (Schierstaedt et al., 2019). While *Listeria monocytogenes* has a good rate of survival in soil, it is also inhibited by the soil microbiota (McLaughlin et al., 2011). On the other hand, even if human pathogens fail to establish themselves, they may still cause shifts in microbial communities, as has been observed for *E. coli* (Mallon et al., 2018). This phenomenon has been described in Mallon et al. (2018) by the term 'legacy effect'. In the case of human pathogens, this would mean that irrigation with contaminated water would first result in changes to the microbial community and then, in a second stage following the repeated application of irrigation water, this would ultimately result in successful colonisation by the human pathogens.

All in all, the interplay of biotic and abiotic factors in soil is a very complex affair. This interplay is also influenced by a range of agricultural processes and activities. It is therefore exceedingly difficult to estimate the persistence of human pathogens in agricultural soils and make predictions about the same. As a rule of thumb, even low levels of human pathogens in soil are sufficient to colonise plants, which then may act as potential sources of infection for humans.

3.1.3.2 Uptake, survival and propagation of *Salmonella*, STEC and *Listeria monocytogenes* in plants

Plants are a natural habitat for human pathogens such as *Salmonella* (Brandl et al., 2013, Fletcher et al., 2013). As a general rule, the natural microbiome of plants and the soil prevents human pathogens from establishing themselves in the first place. Human pathogens colonise our crops on their surfaces or invade the plant tissue, where they then propagate themselves systemically (Brandl et al., 2013, Hernandez-Reyes and Schikora, 2013, Schikora et al., 2012a, Schikora et al., 2012b)—always assuming that they can successfully compete with the autochthonous microbial community. This ambient microbiota can reduce the growth of STEC on spinach leaves, for example (Huang, 2012). However, there are also indications that human pathogens can be protected from environmental factors by the biofilm of the plant's own microbiota, thereby gaining increased environmental resistance (Olaimat and Holley, 2012, Vogeleer et al., 2014). For *E. coli* O157:H7, these kinds of biofilms have been detected after only 24 hours on parsley and lettuce leaves (Niemira and Cooke, 2010, Patel et al., 2011). Field trials indicate that *E. coli* O157:H7 can survive for up to 177 days on parsley and at least 25 days on lettuce leaves (Islam et al., 2004, Solomon et al., 2003, Zhang et al., 2009a).

On the other hand, plant pathogens can promote the colonisation of human pathogens in and on plants as a result of the greater availability of nutrients from infected plant cells. Moulds can also support the penetration of human pathogens into the plant and their subsequent growth as a result of shifts in pH (Brandl, 2006).
While some species of plant are susceptible to colonisation with *Salmonella*, and even enable the entry and translocation of the pathogen into the plant, other species exhibit resistance (Golberg et al., 2011, Guo et al., 2001, Klerks et al., 2007). As a general rule, hydroponic cultivation makes plants more susceptible to colonisation with human pathogens than cultivation in soil (Hirneisen et al., 2012, Riggio et al., 2019, Warriner et al., 2003).

3.1.3.2.1 Colonisation

Human pathogens are capable of adhering to plant surfaces or actively colonising the interior of the plant (Garcia et al., 2014, Schikora et al., 2011, Schikora et al., 2012a, Shirron and Yaron, 2011). In the case of external colonisation, human pathogens applied to plants were detectable weeks and months later, although generally only at very low levels.

The colonisation of plants with human pathogens depends on a wide number of factors, including the plant species (Darlison et al., 2019), soil type (Jechalke et al., 2019), temperature and humidity. *Salmonella* can also survive on dry plant surfaces, and then propagate if warm and moist conditions should return (Brandl, 2006).
The type of irrigation method used also influences opportunities for colonisation. With below-ground irrigation of white radish sprouts with reclaimed waste water, the increase in faecal indicator microbes (faecal coliforms and *E. coli*) in plants was lower than with above-ground irrigation (Balkhair, 2016). With above-ground irrigation using reclaimed waste water, another factor is the plant habit, as studies carried out in Morocco have shown. Vegetables that had direct contact with water or soil (e.g. cucumbers, alfalfa) contained significantly larger quantities of faecal coliforms (El Hamouri et al., 1996) and were more often contaminated with *Salmonella* than tomatoes (Melloul et al., 2001).
Yet even when above-ground drip irrigation is used, there is still the possibility of human pathogens being transmissible from soil to open-air vegetable crops in the event of heavy rainfall. In Portugal, lettuce plants were irrigated with reclaimed waste water containing approximately 10^3 *E. coli* and 1 *Salmonella* per 100 ml. After rainfall, the concentration of *E. coli*

in the lettuce plants rose by more than two log levels to over 6.5×10^4 CFU/g and *Salmonella* was also detectable on the lettuce leaves (Bastos and Mara, 1995).

The fertiliser used can also influence the colonisation of crops by human pathogens. Chicken pellets lead to a higher colonisation rate on spinach plants, for example (Shah et al., 2017), although no influence on the colonisation rate of plants was observed for poultry litter and pig slurry (Jechalke et al., 2019). Interestingly, the survival of *E. coli* O157:H7 on plants was much improved if the bacteria had previously been adapted to harsh environmental conditions by passing through the digestive tract of cattle (Semenov et al., 2010). This could imply that the *E. coli* distributed in fresh cattle manure can survive longer and more effectively on crops than in association with other organic fertilisers. By way of comparison, *Salmonella* survived longer in soil used to cultivate butterhead and lamb's lettuce if the pathogen had been inoculated previously by poultry litter or pig slurry in the soil, and so leading to a higher crop colonisation rate (Jechalke et al., 2019, Justus-Liebig-Universität Gießen, 2018).

Compared with external colonisation, internal colonisation is a process whereby bacteria invade the plant via its stomata, trichomes, roots and surface wounds (Berger et al., 2010, Lapidot and Yaron, 2009, Saldana et al., 2011, Wiedemann et al., 2015, Zhang et al., 2009a, Zhang et al., 2009b). As has been shown by the *plantinfect* project (Justus Liebig University Giessen, 2018), all three *Salmonella* strains tested (*S.* Typhimurium strain 14028s, *S.* Typhimurium strain LT2 and *S.* Senftenberg) colonised both butterhead and lamb's lettuce. It is presumed that the *Salmonella* strains entered via the roots and then spread within the plant via the plant's vascular system. According to Dong et al. (2003), *Salmonella* appear to preferably colonise secondary roots, including root hairs, since these offer a good source of nutrients and entry point.

While the internalisation of *E. coli* O157:H7 from contaminated soil was demonstrated in various types of lettuce plants, uptake of the bacterium did not occur in basil plants (Chitarra et al., 2014). Other authors have been able to show that STEC are taken up by wheat plants during the wheat seed germination and growth process (Martinez et al., 2015). An uptake depth of 10–200 µm under the plant surface has been described, which makes both decontamination (e.g. by washing techniques) and potential pathogen detection significantly more difficult (Beuchat and Ryu, 1997, Solomon et al., 2002).

Warriner et al. (2003) investigated the intake of *E. coli* by young spinach plants. In hydroponic cultivation with approx. 100 *E. coli* per ml, the strain was already detectable in the root interior by day 16. On cultivation in contaminated soil, however, the strain was detectable in the root interior only on day 32, when the *E. coli* concentration had risen from around 100 CFU/g by roughly three log levels. It is possible that competing soil bacteria prevent the uptake of *E. coli* into the roots. Detection in samples from spinach leaves was only sporadically successful and could have been caused by cross-contamination. Accordingly, the authors estimate that there is only a low probability of *E. coli* occurring inside the edible portion of spinach leaves.
Another group of researchers showed that *Salmonella* is detectable after only one day in the lower part of tomato plants cultivated hydroponically, and from the third day onwards in increasing quantities in their leaves, following irrigation of these plants with diluted *Salmonella* cultures (approx. $\log_{10}$ 4.5 CFU/ml). On day 9, *Salmonella* was quantitatively detectable in all parts of the plant ($\geq \log_{10}$ 3.38 CFU/g), with *S.* Montevideo predominant (Guo et al., 2002).

Uptake of *Listeria monocytogenes* into plants from the soil or other substrates used for cultivation has been shown in a study on lettuce plants. Internalisation in this case was dependent on the cultivation temperature. The pathogen was still detectable after 80 days at 24 °C but

not at 30 °C. The inoculum used in this study (10^7 CFU/ml) was comparatively high, however. The same study demonstrated no uptake of *Listeria monocytogenes* by basil plants (Chitarra et al., 2014). Other studies with lettuce plants (Murphy et al., 2016), and with wheatgrass and barley (Kutter et al., 2006), were merely able to demonstrate surface contamination of the plant, but no uptake of *Listeria monocytogenes* into the plants. Other studies with butterhead lettuce have also drawn attention to the possibility of internalisation. In a glasshouse experiment, *Listeria monocytogenes* was found in internal tissue layers when seed had been inoculated 20 days earlier (Shenoy et al., 2017).

The hydroponic cultivation of vegetable plants seems to promote the uptake of *Listeria monocytogenes* significantly. A study by Hofmann et al. (2014) has shown that, alongside *Salmonella*, *Listeria monocytogenes* is also capable of colonising the roots of spinach and lamb's lettuce. In this study, the pathogen was primarily detected in spinach in the root hair zone intercellular space and not on the root hairs or root tips. In lamb's lettuce, *Listeria monocytogenes* was detected just behind the root tip and, more rarely and at lower levels, in the intracellular space of older root sections. In contrast, *Listeria monocytogenes* was capable of colonising plants via contaminated soil only in a few cases during the same study. Similar results have been observed with butterhead lettuce in hydroponic systems (Standing et al., 2013). In this study, the authors detected colonisation both with *Listeria monocytogenes* and *S. enterica* serovar Typhimurium for up to 28 days in the hydroponic system.
A study by Koseki et al. (2011) showed that spinach roots in hydroponic cultivation at high inoculant levels (10^6 CFU/ml) were more likely to be colonised by *Listeria monocytogenes* than at low inoculant levels (10^3 CFU/ml). In a comparison with *S.* Typhimurium, *S.* Enteritidis and *E. coli* O157:H7, however, *Listeria monocytogenes* demonstrated a much lower rate of colonisation (0.3x). Colonisation rates via the roots were seven times higher than via the leaves.

The ability of human pathogens to colonise plants internally is strongly dependent on the pathogen species and/or isolate. Accordingly, various *S. enterica* serovars differ markedly in their plant colonisation capabilities. The reason for this is probably differences in the flagellin protein formed by *Salmonella*, which triggers varying defensive reactions on the part of the plants. In contrast, *S. enterica* serovars have developed variants that can no longer be identified by the plant and which therefore simplify endophytic colonisation.

Lapidot and Yaron (2009) were able to show that *Salmonella* Typhimurium strains lacking the capability for biofilm formation were able to invade parsley plants from contaminated soil only at low levels. The experiments established a pathogen concentration of around two log levels higher in the soil, and a slightly lower pathogen content in the stalks and leaves of the parsley plants. Various studies also point to differences between strains in terms of internalisation capabilities for STEC and *E. coli* (Erickson et al., 2019, Wright et al., 2013). With *E. coli* O157:H7, colonisation is conditional on certain adherence factors (Eißenberger et al., 2018) and (to an extent) on flagella and pili (Saldana et al. (2011).

Similarly to plant pathogenic bacteria, human pathogens also appear to require the type III secretion system (T3SS) for successful colonisation of stomata and leaves. The transformation of an *E. coli* strain with a plasmid-coded T3SS enabled this strain to achieve colonisation and internalisation (Saldana et al., 2011).

Differences between strains in terms of their ability to colonise plants is also suspected for *Listeria monocytogenes*. Kljujev et al. (2018) investigated the potential colonisation of the roots of various vegetable plants (butterhead lettuce, spinach, carrots, celery, tomato, sweetcorn) and parsley in a hydroponic system by the strains EGD-e (serotype 1/2a) and SV4B (serotype 4b). They were able to show that the EGD-e strain preferably colonised the

roots of carrots, parsley, celery and sweetcorn, both on the surface and endophytically (intercellular space). The SV4B strain preferably colonised leafy vegetables such as butterhead lettuce and spinach but tomatoes, carrots, parsley and celery to a lesser extent. The exact mechanisms of colonisation have not been investigated to date.

The internal colonisation rate fluctuates strongly depending on the host plant, the human pathogen species, the type of inoculation and numerous other factors. Many studies indicate that internalisation is a rare occurrence or takes place only under extreme conditions (Erickson et al., 2019, Olaimat and Holley, 2012, Wright et al., 2013). In the *plantinfect* project (Justus Liebig University Giessen, 2018), the internal colonisation rate for various *Salmonella* strains was 0.7–1.4% for butterhead lettuce and 0.3–0.4% for lamb's lettuce (Jechalke et al., 2019). Honjoh et al. (2014) found *Salmonella* in 2.9% of the lettuce leaves that had been surface-sterilised. Significantly higher internal colonisation rates were also reported, however. The internal colonisation rate of romaine lettuce 22 days after soil application of 10^8 CFU per plant was 94% for *Salmonella* and 68% for STEC (Nicholson et al., 2015). Gu et al. (2018) obtained an internal colonisation rate of 50% in the plants and 1% in the fruit following the irrigation of tomatoes with water contaminated with *Salmonella* (10^4 CFU/ml).

In relation to the internal colonisation of plants, human pathogens seem to adopt similar infection strategies as in animals. This similarity is at least suggested by the high degree of correspondence between the *Salmonella* transcriptome after the colonisation of lettuce and coriander plants and the *Salmonella* transcriptome after the infection of animals (Goudeau et al., 2013).

3.1.3.2.2 Propagation and migration in plants

Once internalised in the plant, human pathogens adopt very different strategies for their subsequent propagation and spread. The survival of human pathogens in plants is influenced by a wide variety of genetic factors. The 43 *Salmonella* strains tested by Wong et al. (2019) differ significantly in terms of their survival in sprouted lettuce and tomato seeds. One possible explanation for the differences within the *S. enterica* species could be the plasmid-mediated virulence of *Salmonella* (*spv* region) (Boyd and Hartl, 1998) or, alternatively, variations in adhesin availability (Hansmeier et al., 2017).
The propagation of human pathogens is also strongly dependent on the specific plant species. Although no increase in bacterial count could be determined in the leaves of lettuce plants and spinach following infiltration with the *E. coli* O157:H7 Sakai isolate, bacterial propagation of >400x was observed after corresponding infiltration of leaves with *Nicotiana benthamiana* (Wright et al., 2017). The reason for this is presumably variations in host plant preferences or differences in the plant defence system.
Brandl (2008) and Brandl and Amundson (2008) showed that *E. coli* O157:H7 and *Salmonella* can propagate on the leaves of lettuce plants, with leaf age being a significant determinant of the extent of colonisation.

The spread of human pathogens within the plant is not at all uniform. Alongside intensively colonised regions, regions with no colonisation at all are commonly found. The spatial colonisation of the roots of various plants, including lettuce, can be readily visualised with the aid of an FISH-CLSM analysis (fluorescence in situ hybridisation-confocal laser scanning microscopy), for example, as shown by Kljujev et al. (2018) for *S*. Typhimurium LT2, *S*. Typhimurium 14028s and *S*. Typhimurium S1.

3.1.3.2.3 Propagation in plant-based foods

Listeria monocytogenes is particularly capable of propagating on those kinds of plant-based foods that do not contain any antimicrobial constituents, and which—depending on their processed state, storage temperature and storage duration—offer the pathogen beneficial growth conditions in terms of nutrients, water availability and pH. This is the case for leaf lettuce and leafy vegetables, for example (Hofmann et al., 2014, Oliveira et al., 2010).
In one study, *Listeria monocytogenes* was shown to achieve better growth in the plant-based products investigated than *Salmonella* (Sant'Ana et al., 2012). These results are readily explained by the known cold-tolerant behaviour of *Listeria monocytogenes* (Gandhi and Chikindas, 2007). The study by Sant'Ana et al. (2012) provides a good overview, showing that both *Listeria monocytogenes* and *Salmonella* were capable of growth in the plant-based products tested, and particularly at a slightly elevated storage temperature of 15 °C. As already summarised by Delaquis et al. (2007), previous studies have identified storage temperature as the primary influential factor on the development of *E. coli* O157:H7 in packaged, ready-to-eat lettuce. *E. coli* O157:H7 appears capable of survival and growth in all variations of packed leafy vegetables at storage temperatures of $\geq$8 °C (Delaquis et al., 2007).

With all of the research results presented here, it must be remembered that most of the investigations that aimed to detect human pathogens on or in plants were completed using conventional culturing methods. These methods do not take into account the occurrence of VBNC cells, i.e. viable but non-culturable cells. The significance of such VBNC cells was demonstrated by the EHEC/EAEC O104:H4 outbreak in 2011 (Aurass et al., 2011).

The low abundance of human pathogens in or on plants but also the occurrence of VBNC cells presents diagnostics with a significant challenge. Accordingly, the molecular culture-independent methods are not sensitive enough (Blau et al., 2018). Often, enrichment methods are necessary in order to detect human pathogens on fresh products. Nonetheless, even a few cells can propagate rapidly in suitable circumstances (nutrients, temperature), as has been shown by the non-selective enrichment of cilantro, rocket and mixed salad in peptone water (Blau et al., 2018).
The isolation of STEC from plant-based foods is also hindered by the abundance of background microbiota, which includes *Pseudomonas* spp. and other *Enterobacteriaceae*, including non-pathogenic *E. coli*, which can be present in numbers between 10^6 CFU/g and 10^9 CFU/g (Tzschoppe et al., 2012). Plant lysates can also influence the culturability of STEC (Thao et al., 2019) and thus make isolation more difficult.

3.1.3.3 Occurrence of *Salmonella* in plant-based foods

Investigations made by the food control authorities in Germany indicate that foods of plant origin in Germany are only rarely contaminated with *Salmonella*. An evaluation made by the Federal Office of Consumer Protection and Food Safety (BVL) of regulatory microbiological testing conducted in the German federal states on fresh leafy vegetables and leafy vegetable products/preparations between 2014 and 2016 showed that *Salmonella* were detectable in 2 (0.2%) of 1,009 samples tested from fresh leafy vegetables (basil, leaf lettuce) and in 1 (0.2%) of 408 samples tested from leafy vegetable products/preparations (dried leafy vegetables).

In 2016, 367 samples of fresh sprouts were tested as part of zoonosis monitoring, of which 3 samples (0.8%) tested positive for *Salmonella*, while no *Salmonella* was found in a total of 480 tomato samples (Hartung et al., 2019).

In frame of the Federal Control Plan (Bundesweiter Überwachungsplan - BÜp) in Germany in 2017, no *Salmonella* was detectable in 304 samples tested from desserts and mixed milk products using fresh or thawed fruits, nor in 138 samples tested from unpasteurised smoothies containing vegetable and/or fruit ingredients (BVL, 2018).

In two research projects conducted by the MRI, a total of 884 separate plant-based products were tested in recent years. These included 600 samples from conventional and organic cultivation, which were taken from food retailers in northern and southern Germany during the years 2015 and 2016 (115 kitchen herbs, 40 cucumber samples, 79 carrot samples, 80 butterhead/lamb's/loose-leaf lettuce, 116 ready-to-eat salads, 81 samples of mushrooms and 89 samples of sprouts) (Becker et al., 2019). In the product group 'Ready-to-eat mixed salads', *Salmonella* (2x *S.* Enteritidis, 1x *S.* Szentes) was detected in 3 of 116 samples tested, equating to a prevalence of 2.8%. Tests on butterhead/lamb's/loose-leaf lettuce samples (N = 80) detected *S.* Enteritidis in one sample (1.3%) of butterhead lettuce.

In 2016 and 2017, further tests were conducted on a total of 244 product samples (butterhead/lamb's/loose-leaf lettuce, endive, chicory, carrots, cucumbers, etc.) taken directly from a processing company in southern Germany, as well as another 40 retail samples. No *Salmonella* was detected in these samples (Kabisch et al., 2017).

In 2018, the National Reference Laboratory for *Salmonella* at the BfR received a total of 4,807 *Salmonella* isolates for typing from testing laboratories in Germany. Only 17 of these isolates (0.35%) originated in food of plant origin. In 2017, the equivalent figure was 8 (0.20%) from 3,873 isolates. Food matrices from which these *Salmonella* were isolated are listed in Table 5.

Table 5: *Salmonella* serovars of the *Salmonella* isolates sent to the NRL from food of plant origin (2017–2018)

2017		2018	
Serovar	Matrix	Serovar	Matrix
S. Bracknell	Oat flour	*S*. Agona	Vegetable (carrot salad)
S. Coeln	Rye flour (2x)	*S*. Typhimurium	Vegetable crisps Mixed salad
S. Infantis	Herbs	*S*. Douala	Sesame
S. Oranienburg	Fruit juice	*S*. Mbandaka	Sesame
S. subspec. I. 11:z41:e,n,z15	Sesame seed	*S*. Mishmarhaemek	Sesame
S. subspec. II. 55:k:z39	Vegan spelt pasta	*S*. Plymouth	Sesame
S. subspec. IV. 43:z4, z23	Basil	*S*. subspec. I. 1,3,19:z:-	Spices
		S. Oranienburg	Spices
		S. Weltevreden	Mushrooms
		S. Louisiana	Banana leaves
		S. Faji	Banana leaves

In the 2016 Dutch National Control Plan, more than 3,457 products were tested (raw plant-based goods). *Salmonella* was detected in only one sample (0.02%). In the same context, lettuce/leafy vegetables and fresh herbs were also analysed. While *Salmonella* could not be detected in the 300 samples from lettuce/leafy vegetables, it was detected in 4.6% of fresh herb samples (Heythuyzen, 2016). The Dutch National Institute for Public Health and the En-

vironment (RIVM) was able to detect *Salmonella* in only 1 (0.38%) of 1,860 samples of lettuce and vegetables tested, as well as in 1 (0.17%) of 1,151 samples tested of ready-to-eat mixed salads, as taken from the premises of two manufacturers during the period 2006–2007. Data from the Danish Zoonosis Report for 2017 reveal that *Salmonella* was not detectable in samples of lettuce, spinach, sprouts or herbs (N = 94) (Anonymus, 2018).

If one compares the national/European data with data from North America, it is clear that the detection rate of *Salmonella* in plant-based fresh products is also found to be in similar percent ranges, typically of under 1% (Table 6).

Table 6: Detection of *Salmonella* in vegetables in North America

Country	Year	Food	Positive results/total number of samples	Proportion of positive samples in % (95% confidence interval)	Literature
North America					
Canada	2009 to 2013	Herbs (parsley, coriander, basil, dill, mint, etc.)	5/6,027	0.08 (0.04–0.19)	Denis et al. (2016), Reddy et al. (2016)
		Leafy vegetables (loose-leaf lettuce, butterhead lettuce, spinach, etc.)	2/11,400	0.02 (0–0.06)	
		Green onions	1/2,963	0.03 (0.01–0.19)	
		Tomatoes	0/4,416	0 (0–0.09)	
USA	2002 to 2012	Coriander	31/9,245	0.34	Reddy et al. (2016)
		Parsley	5/1,700	0.29	
		Sprouts	32/12,976	0.25	
		Spinach	22/11,030	0.20	
		Green onions	6/7,332	0.08	
		Butterhead lettuce	7/10,816	0.06	
		Bagged salads	3/7,269	0.04	
		Tomatoes	5/24,669	0.02	

Virtually no quantitative data are available on the occurrence of *Salmonella* in various plant-based foods. Nonetheless, Pielaat et al. (2008) reported bacterial counts of 0.0019 and >0.281 *Salmonella* per gram in lettuce/vegetable samples and in pre-cut packaged lettuce.

Da Silva Felicio et al. (2015) developed a semi-quantitative model in order to determine and categorise the interaction of certain pathogens with specific foods of non-animal origin in the EU. The combinations receiving the highest scores were *Salmonella*/leafy vegetables as well as *Salmonella*/bulb and stem vegetables, followed by *Salmonella*/tomatoes and *Salmonella*/melons. In their investigations of the occurrence of *Salmonella* in plant-based fresh products, Reddy et al. (2016) show that the highest levels of *Salmonella* prevalence occurred in coriander (0.34%), followed by parsley and spinach (0.29%), chili peppers (0.26%) and sprouts (0.25%). Reddy et al. (2016) argue that fertiliser, wet manure, soil, irrigation water or animals could be potential sources of this *Salmonella* contamination. Accordingly, soil and water could be potential risk factors on contact with the undersides of the leaves; vegetables that grow nearer to the ground must therefore be more susceptible to contamination. The authors argue that this hypothesis is substantiated by the data they provide on the prevalence of *Salmonella* in coriander, spinach and parsley (Reddy et al., 2016).

3.1.3.4 Occurrence of STEC in plant-based foods

STEC have already been isolated from a variety of plant-based foods, which include cabbage, celery, coriander and cress (Olaimat and Holley, 2012).

Investigations of the food control authorities in Germany indicate that fruit and vegetables are only very rarely contaminated with STEC. An evaluation of data provided by the Federal Office of Consumer Protection and Food Safety (BVL) on routine microbiological testing in the German federal states of fresh leafy vegetables and leafy vegetable products/preparations between 2014 and 2016 was conducted. During this period STEC were detected in 3 (0.3%) of 931 samples tested from fresh leafy vegetables (rocket). No STEC was found in 161 samples of leafy vegetable products/preparations tested.

As part of zoonosis monitoring in 2012, STEC were detected in 4 (1.3%) of 312 samples of loose-leaf and butterhead lettuces tested, which were sampled directly from the producers. In 464 samples taken from the retail segment during the same year, no STEC were detectable, however (BVL, 2014). In the following years, no STEC were detected in samples of fruit and vegetables tested as part of zoonosis monitoring (Table 7).

Table 7: STEC in fruit and vegetables in Germany, 2012–2016, zoonosis monitoring

Year	Food	Number of samples analysed (N)	Positive samples (n)	Proportion of positive samples in %
2012	Loose-leaf/butterhead lettuce from producing company	312	4	1.3
	Loose-leaf/butterhead lettuce from retail	464	0	0.0
2013	Fresh strawberries from producing company	336	0	0.0
	Fresh strawberries from retail	424	0	0.0
2014	Fresh herbs	426	0	0.0
2015	Pre-cut loose-leaf lettuce	383	0	0.0
2016	Tomatoes (cocktail, cherry)	475	0	0.0
	Sprouts (fresh)	368	0	0.0

In 2017, STEC were detected in just 1 of 155 samples taken in frame of the Federal Control Plan from smoothies made from fruit or fruit and vegetables (BVL, 2018). In contrast, no STEC were found in 28 samples of freshly pressed vegetable juice taken from juice bars in 2012 (BVL, 2013).

As part of two research projects within 2015 to 2017, surveys concerning the presence of pathogenic microorganisms in fresh products were conducted at the Max Rubner Institute. In these tests on retail samples (N = 200) performed in 2015, *E. coli* O26:H11 was detected with the three gene markers *stx1, stx2* and *eae* in one sample of pre-cut mixed lettuce (Fiedler et al., 2017). In 2016, a further 115 samples (fresh mushrooms, carrots, sprouts) were analyzed and *E. coli* O146:H28 with the pathogen marker gene *stx2* was isolated from one sample (carrots).

In 2016 and 2017, as part of the second project, a total of 244 product samples (butterhead/lamb's/loose-leaf lettuce, endive, chicory, carrots, cucumbers, etc.) were taken directly at a processing company in southern Germany and then analysed. *E. coli* O146:H28 (*stx2*-

positive) was isolated from one sample (Kabisch et al., 2017). Another 40 retail samples of lettuce and mixed salads were tested negative for STEC in 2017.
Overall, following tests made on 599 fresh product samples, STEC was detected with a prevalence of 0.5%.

In one Dutch survey, a total of 0.11% of the plant-based raw products tested were contaminated with *E. coli* O157 (Wijnands et al., 2014). In the period from August 2013 to March 2016, the 'Food Compass' monitoring study performed in the Netherlands established an STEC prevalence of 0.2% from raw goods monitoring (N = 3,013) (Heythuyzen, 2016). During testing of fresh herbs (basil and coriander) conducted in Belgium, the detection of STEC via PCR, defined as the occurrence of the genes *stx* and *eae*, was positive in 11 of 592 samples. Although it was not possible to isolate STEC from of these PCR-positive samples (Delbeke et al., 2015).

As a result of the rare detection of STEC in fresh fruit and vegetables, STEC also occur rarely in notifications to the European Rapid Alert System for Food and Feed (RASFF), as can be seen from Table 8.

Table 8: Notifications made to the EU rapid alert system (RASFF) following STEC/EHEC detection in fruit and vegetables (11/2014–5/2019)
Source: https://webgate.ec.europa.eu/rasff-window/portal/: 1 Jan 2014 to 21 Aug 2019)

Date	Notifying country	Topic (original text)
24 May 2019	Germany	shigatoxin-producing *Escherichia coli* (stx1+ stx2+ eae+) in organic baby spinach from Italy
22 Oct 2018	Germany	shigatoxin-producing *Escherichia coli* (stx2+ /25g) in sprouts from Germany, with raw material from Italy
9 Oct 2018	Netherlands	shigatoxin-producing *Escherichia coli* (stx2f+ /25g) in chilled chopped endive from the Netherlands
22 Sep 2017	Finland	shigatoxin-producing *Escherichia coli* (stx2+) in lamb´s lettuce (Valerianella locusta) from Italy
3 Aug 2017	Germany	shigatoxin-producing *Escherichia coli* (stx2+ /25g) in beetroot sprouts from the Netherlands
28 Apr 2016	Netherlands	shigatoxin-producing *Escherichia coli* (stx1+ /25g) in fresh bean sprouts (tauge) from the Netherlands
7 Sep 2016	Finland	foodborne outbreak suspected to be caused by shigatoxin-producing *Escherichia coli* (stx+, eae+, 3100 CFU/g) in rucola from Denmark, via Sweden
28 Jul 2015	Finland	shigatoxin-producing *Escherichia coli* (stx1-/stx2+/eae- /25g) in lentil sprouts with raw material from Canada, packaged in Sweden
7 Jul 2015	Netherlands	shigatoxin-producing *Escherichia coli* (VTEC: stx1) in sprouted beans from the Netherlands
27 May 2014	Czech Republic	shigatoxin-producing *Escherichia coli* (presence /25g) in cherry tomatoes from Morocco, via France
25 Nov 2014	Denmark	shigatoxin-producing *Escherichia coli* (vtx+ and eae+) in dates from Iran, via Sweden

In the USA, Zhang et al. (2019) investigated the microbiological status of leafy vegetables (iceberg and romaine lettuce, spinach) and sprouts from 2009 to 2014. For leafy vegetables (N = 14,183), prevalences of 0.01% and 0.07% were detected for *E. coli* O157:H7 and non-O157 STEC, respectively. While *E. coli* O157:H7 was not detected on sprouts, 0.04% of the sprouts examined (N = 2.652) tested positive for non-O157 STEC.
In the USA, Korir and colleagues (Korir et al. (2016)) analyzed a total of 414 fresh products and were able to isolate *E. coli* O157:H7 from one sample of fresh spinach.

In a further study conducted in the USA between 2002 and 2012, a total of 132 STEC strains were isolated from samples of plant-based fresh products. This equates to a prevalence of 0.5%. Most (52%) of these STEC strains occurred in samples of fresh spinach, followed by loose-leaf lettuce (21%) and coriander (14%). Aside *E. coli* O157:H7, other serotypes associated with humans disease (O121: H19, O26:H11 and O165:H25) were also detected in these samples. Serotyping for 56 of these 132 STEC strains was only partially possible or not possible (Feng and Reddy, 2013).

3.1.3.5 Occurrence of *Listeria monocytogenes* in plant-based foods

As a result of its ability to adopt a saprophytic lifestyle, *Listeria monocytogenes* can survive very well in soil, plant remains and waste water on land that is used for agriculture. Entry of the pathogen into soil and surface waters can occur as a result of for example the application of organic fertilisers (wet and solid manure) in agriculture, from agricultural waste water or from wild animals (Dowe et al., 1997, Ivanek et al., 2006). Plants growing in or near to the soil can therefore be contaminated with *Listeria monocytogenes* by the direct adhesion of soil particles, or as a result of rain or irrigation. Very frequently, the food is not contaminated with the pathogen until processing, owing to an associated lack of operational hygiene. The contamination of plant-based foods with *Listeria monocytogenes* is therefore generally possible both before the harvest as well as during processing and packing. While the washing of plant-based foods can help to reduce surface contamination from *Listeria monocytogenes* (Hofmann et al., 2014, Pezzuto et al., 2016), the effectiveness of this washing depends on the plant species and the characteristics of its surfaces (Nastou et al., 2012).

In terms of the occurrence of *Listeria monocytogenes* in plant-based foods in Germany, few data are available, and these do not permit a valid and comprehensive estimate. However, they do indicate that the pathogen occurs in and on a wide variety of plant-based foods, as reports from zoonosis monitoring in Germany and surveys by the BfR have shown (BVL, 2013, BVL, 2014, BVL, 2015, BVL, 2016, BVL, 2016, BVL, 2017, BVL, 2018, Hartung, 2010, Hartung and Käsbohrer, 2011, Hartung and Käsbohrer, 2012, Hartung and Käsbohrer, 2013, Hartung et al., 2018, Hartung et al., 2014, Hartung et al., 2015, Hartung et al., 2016).

Table 9 presents qualitative detection data from routine sampling of vegetables and lettuce for the categories ‘Lettuce, leafy vegetables, sprouts, fresh vegetables for raw consumption (excluding leafy, chopped and sprouts)’, ‘Fresh vegetables excluding rhubarb’ and ‘Pre-cut vegetables and lettuce’ for 2008 to 2016. The pathogen has been regularly detected in a few samples. However, due to the limited and widely varying scope of annual sampling, no valid statements on prevalence can be derived from these data.

As part of zoonosis monitoring in 2012, loose-leaf and butterhead lettuce from producing companies (N = 300) and retail (N = 422) was sampled, while pre-cut loose-leaf lettuce from retail (N = 344) was investigated in 2015. Detection rates here were 3.7%, 2.6% and 2.0% (Table 10). No sample exhibited a concentration of *Listeria monocytogenes* of more than 100 CFU/g (Table 11). In 2016, fresh cocktail and cherry tomatoes, as well as fresh sprouts, were also investigated qualitatively for the occurrence of *Listeria monocytogenes*. While the pathogen was not detected in tomatoes, 1.8% of the samples of fresh sprouts tested were contaminated with *Listeria monocytogenes*. *Listeria monocytogenes* was not detected by quantitative testing in any sample of sprouts (limit of detection <10 CFU/g).
There is also a paucity of data for the detection of *Listeria monocytogenes* in fruit in Germany over the last few years. For the period 2011 to 2016, samples of both fresh fruit and fruit salads (pre-cut) have occasionally tested positive (Table 9). These data do not permit any estimate about the prevalence of the pathogen, however.

As part of zoonosis monitoring, previous testing for the occurrence of *Listeria monocytogenes* has only covered fresh strawberries from producing companies (N = 300) and retail (N = 463) in 2013. Similar rates of detection were reported for both types of origin (1.3% and 1.1%, see Table 10).

In 2012 and 2017, freshly pressed fruit and vegetable juices from juice bars (sold unpackaged) and/or unpasteurised smoothies made from fruit were tested for *Listeria monocytogenes* as part of Germany's Federal Control Plan. The pathogen was detected using molecular biology methods in only one sample from 2012 (BVL, 2013, BVL, 2018).

There are no valid data on the occurrence of *Listeria monocytogenes* in herbs in Germany. In international studies, *Listeria monocytogenes* could not be detected on a wide range of fresh and dried herbs, including lemon balm, sage, mallow, camomile, dill, parsley, mustard and coriander (FSAI 2015, Johannessen et al., 2002, Johnston et al., 2005, Korir et al., 2016, Vitullo et al., 2011). In contrast, antimicrobial effects have been proven for many essential oils, with some efficacy also noted against *Listeria monocytogenes* (de Carvalho et al., 2015, Lopez et al., 2005). According to a notification made to RASFF in 2016, *Listeria monocytogenes* was detected at a concentration of 90 CFU/g in a sample of fresh thyme two days before the expiry date. During the same year, official food controls in Germany detected *Listeria monocytogenes* in a sample of pre-cut frozen parsley.

In two research projects conducted by the MRI, a total of 884 separate plant-based products were tested in recent years. These included 600 samples from conventional and organic cultivation, which were taken from food retailers in northern and southern Germany during the years 2015 and 2016 (115 kitchen herbs, 40 cucumber samples, 79 carrot samples, 80 butterhead/lamb's/loose-leaf lettuce, 116 ready-to-eat salads, 81 samples of mushrooms and 89 samples of sprouts) (Becker et al., 2019). An excerpt of the results from this testing is presented in Table 12. The proportion of positive samples here ranged from 0% to 2.6%.

In a second MRI research project, tests for the occurrence of *Listeria monocytogenes* were conducted on 244 product samples (butterhead/lamb's/loose-leaf lettuce, endive, chicory, carrots, cucumbers, etc.) taken directly from a processing company in southern Germany. This testing detected *Listeria monocytogenes* in 1 of 10 samples of iceberg lettuce, in 3 of 15 samples of loose-leaf lettuce of the Lollo Rosso variety and in 1 of 14 samples of loose-leaf lettuce of the Lollo Bionda variety. *Listeria monocytogenes* was not detectable in a further 40 retail samples of lettuce and mixed salads from 2017.

Table 9: *Listeria monocytogenes* in fruit and vegetables in Germany, 2008–2016, qualitative testing of routine samples

Food	Year	Samples tested (N)	Positive samples (n)	Proportion of positive samples in %	95% confidence interval
Lettuces	2011	34	2	5.88	0.00–13.79
	2012	157	3	1.91	0.00–4.05
	2013	49	1	2.04	0.00–6.00
	2014	36	1	2.78	0.00–8.15
	2015	78	0		0.00–1.12
	2016	170	5	2.94	0.40–5.48
Leafy vegetables	2011	37	0		
	2012	649	11	1.69	0.70–2.69
	2013	133	3	2.26	0.00–4.78
	2014	210	4	1.90	0.06–3.75
	2015	264	1	0.38	0.00–13.95
	2016	20	1	5.00	0.00–14.55
Sprouts	2011	110	3	2.73	0.00–5.77
	2012	109	6	5.50	1.22–9.79
	2013	53	0		
	2014	35	0		
	2015	105	1	0.95	0.00–2.81
	2016	238	2	0.84	0.00–2.00
Fresh vegetables for consumption raw (excl. leafy, prepared and sprouts)	2011	97	1	1.03	0.00–3.04
	2012	121	2	1.65	0.00–3.92
	2013	59	1	1.69	0.00–4.99
	2014	79	2	2.53	0.00–6.00
	2015	45	3	6.67	0.00–13.95
	2016	252	0		
Fresh vegetables excluding rhubarb	2011	7	0		
	2012	181	3	1.66	0.00–3.52
	2013	38	0		
	2014	104	1	0.96	0.00–2.84
	2015	177	0		
	2016	156	0		
Pre-cut vegetables and lettuces	2008	24	2	8.33	0.00–19.39
	2009	38	1	2.63	0.00–7.72
	2010	7	1	14.29	0.00–40.21
	2013	3	0		
	2015	37	1	2.70	0.00–7.93
Fresh fruit, incl. rhubarb	2011	61	1	1.64	0.00–4.83
	2012	142	0		
	2013	337	1	0.30	0.00–0.88
	2014	67	0		
	2015	96	1	1.04	0.00–3.07
	2016	21	0		
Fruit salads (pre-cut)	2011	56	0		
	2012	94	0		
	2013	49	0		
	2014	65	0		
	2015	70	1	1.43	0.00–4.21
	2016	36	0		

Table 10: *Listeria monocytogenes* in fruit and vegetables in Germany, 2012–2016, zoonosis monitoring, qualitative testing

Year	Food	Number of samples analysed (N)	Positive samples (n)	Proportion of positive samples in % (95 % confidence interval)
2012	Loose-leaf/butterhead lettuce from producing company	300	11	3.7 (2.0–6.5)
	Loose-leaf/butterhead lettuce from retail	422	11	2.6 (1.4–4.7)
2013	Fresh strawberries from producing company	300	4	1.3 (0.4–3.5)
	Fresh strawberries from retail	463	5	1.1 (0.4–2.6)
2015	Pre-cut loose-leaf lettuce	344	7	2.0 (0.9–4.2)
2016	Tomatoes (cocktail, cherry)	478	0	0.0 (0.0–1.0)
	Sprouts (fresh)	271	5	1.8 (0.7–4.4)

Table 11: *Listeria monocytogenes* in fruit and vegetables in Germany, 2012–2016, zoonosis monitoring, quantitative testing

Year	Food	Number of quantitatively tested samples (N)	Number and proportion (%) of positive samples 10–100 CFU/g	Number and proportion (%) of positive samples >100 CFU/g	Microbial content of samples detected >100 CFU/g
2012	Loose-leaf/butterhead lettuce from producing companies	292	0	0	
	Loose-leaf/butterhead lettuce from retail	427	2 (0.5)	0	
2015	Pre-cut loose-leaf lettuce	320	1 (0.3)	0	
2016	Sprouts (fresh)	321	0	0	

Table 12: *Listeria monocytogenes* in various fresh plant-based products that were tested in Germany at the retail level, according to Becker et al. (2019)

Food	Number of samples analysed (N)	Positive samples (n)	Proportion of positive samples in %	Product name	Molecular serogroup
Ready-to-eat mixed salads	116	3	2.6	Mixed lamb's lettuce Mixed salad Garden salad	IIa IIb IVb
Butterhead, loose-leaf/Lollo lettuce varieties	80	1	1.3	Baby lettuce (two colours)	IIa
Sprouts	89	1	1.1	Wok trio	IIa
Mushrooms	81	1	1.2	Enoki mushrooms	IIb
Herbs	115	0	0	–	
Carrots	79	0	0	–	–
Cucumbers	40	0	0	–	–

In the Netherlands, over 3,000 products (raw plant-based goods) were tested for the presence of *Listeria monocytogenes* in 2016. Qualitative positive detection was established in 1.1% of samples tested. In the same context, lettuce varieties/leafy vegetables (N = 157/300) were also analysed, and *Listeria monocytogenes* was found in 4.8% of the qualitatively tested samples (Heythuyzen, 2016).
Zhu et al. (2017) have summarised international studies on the occurrence of *Listeria monocytogenes* in plant-based fresh products. Their work describes prevalences for *Listeria monocytogenes* ranging from 0.9% in sprouts from South Korea (number of samples tested = 112) to 25% in parsley from Malaysia (number of samples tested = 16). It should be noted, however, that studies with high rates of detection in particular typically investigate only low sample counts.

3.1.3.6 Consumption of fresh fruit and vegetables in Germany

The regular consumption of fresh fruit and vegetables is an important part of maintaining a healthy diet.
To estimate the consumption of fresh fruit and vegetables in Germany, survey data was used from the household and fresh food panel maintained by GfK SE. An evaluation of these data was made for the period September 2017 to August 2018, so as to account for seasonal variation during the year.

Consumer data (sales in tonnes) is available to the BfR for the following plant-based foods:

(1) Salad greens and vegetables in the categories salad greens, leafy vegetables, other fresh vegetables that can also be consumed raw (e.g. tomatoes, cucumbers, bell pepper), fresh sprouts (beansprouts and sprouted seeds), and pre-cut vegetables and salads.
(2) Fruit in the categories of fresh fruit, including rhubarb and mixed fruit salad/pre-cut fruit.

In the survey period selected, 2,320,519 tonnes (t) of salad greens and vegetables plus 3,616,843 t of fruit were purchased (Table 13). In the salad greens and vegetables group, the largest category was other fresh vegetables (87.6%), followed by salad greens (9.7%). Leafy vegetables, pre-cut vegetables and salads, and fresh sprouts made up only a small proportion (1.4%, 1.3%, and 0.04%, respectively). Most fruit was consumed as fresh fruit that had not been pre-cut (99.6%).

Table 13: Sales volumes of fresh fruit and vegetables in Germany in tonnes (t) for the survey period 2017/18 (data source: GfK SE, Consumer Panels & Services)

Food	Sales (t)
Salads and vegetables	***2,320,519***
Pre-cut vegetables and salads	30,075
Salad greens	225,376
Leafy vegetables	31,771
Other fresh vegetables, excluding rhubarb	2,032,327
Sprouts – beansprouts and sprouted seeds (fresh)	970
Fruit	***3,616,834***
Fresh fruit, including rhubarb	3,603,722
Mixed fruit salad/pre-cut fruit	13,112

These data are marketing data surveyed at the household level, however. As such, they do not permit any conclusions to be drawn about actual consumption quantities or frequencies, as no information is available about the further use of these foods or food waste generated. To evaluate the relevance of the consumption of raw fruit and vegetables, data for various age groups in the general population were therefore also evaluated in terms of their consumer proportions.
The analysis for adults aged between 14 and 80 is based on data from dietary history interviews conducted for the National Food Consumption Study II (NVSII). This nationwide study was completed from 2005 to 2006 in Germany, and is currently the most representative study of consumption patterns for the German adult population (Krems et al., 2006, MRI, 2008).
To conduct the dietary history interviews, the program 'DISHES 05' was used to interview 15,371 persons retrospectively about their consumption over the last four weeks. These data are particularly suitable for assessing typical consumption patterns.
To ensure that children—a high-risk group—are properly accounted for, consumption data was also taken from the VELS study (Banasiak et al., 2005, Heseker et al., 2003). This nationwide study was carried out from 2001 to 2002 in Germany, covering 816 infants and young children aged from six months to under five years old. Children were considered for the study only if they were no longer being breast-fed (N = 732). The parents logged the food consumed by each child in two nutritional records kept over three consecutive days. Since these data cover a total of six days of consumption, they are suitable for mapping out representative consumption patterns.

The evaluation of these data considered the consumption of raw fruit and vegetables. The analysis excluded entries concerning heated or dried preparations (including preserves and powders) as well as the consumption of food that is only consumed once cooked (e.g. aubergines, broccoli, beans). Consumption entries were therefore included if they specified 'raw, unprocessed'. In the spirit of a conservative approach to the analysis, preparations not further specified were also included, as well as heating methods that do not guarantee the sufficient reduction of microorganisms (e.g. warming and poaching). Frozen products are also included in the evaluation for raw fruit and vegetables, since these are only partially blanched before freezing. While details about the preparation of food are associated with uncertainty in the dietary logbooks, this procedure is therefore more likely to result in an overestimate than an underestimate of the consumer proportions.

The analyses were performed using SPSS version 21.

Table 14 shows the consumer proportions for fruit and vegetables consumed raw for children and adults. A total of 98.1% and 99.8% of adults aged between 14 and 80 consume raw fruit and raw vegetables, respectively[7]. The consumer proportions for children aged between 6 months and <5 years were slightly lower, at 97.5% for fruit and 86.2% for vegetables[8]. A differentiated analysis was completed for infants aged between 6 and <12 months, and for senior citizens aged from 65 years, since these groups are considered to be high-risk groups for foodborne infections. The data reveal that the consumer proportion for raw fruit in the group of children aged under 12 months is almost 100%. Fresh vegetables were consumed by only 55% of those surveyed[8], which is attributable to an improved tolerability of vegetables when cooked for babies and infants. In the from 65 years age group, almost 100% of respondents stated that they had consumed fruit and vegetables raw[7].

[7] Consumed at least once over a four-week reference period.
[8] Consumed at least once over a six-day reference period.

Table 14: Proportion of consumers of raw fruit and vegetables in the German population (source: NVSII(Krems et al., 2006, MRI, 2008); VELS study (Banasiak et al., 2005, Heseker et al., 2003))

	Children overall[a, b]	Adults overall[c]	Children[a, b]		Adults[c]	
	6 months to <5 years	*14 to 80 years*	*6 to <12 months*	*1 to <5 years*	*14 to 64 years*	*≥65 years*
Fruit consumed raw	97.5%	98.1%	98.9%	97.3%	97.8%	99.5%
Vegetables consumed raw	86.2%	99.8%	54.7%	90.9%	99.9%	99.8%

[a] Consumed at least once over a six-day reference period [b] Not breast-fed [c] Consumed at least once over a four-week reference period

3.1.4 Risk characterisation

While the data presented in the current assessment do not permit a valid statement about the occurrence of *Salmonella*, STEC and *Listeria monocytogenes* on or in fresh fruit and vegetables, the data nonetheless indicate that these pathogens have been detectable only rarely and in low quantities in these foods in Germany and in neighbouring EU countries to date. However, the VBNC status of the bacteria could have contributed to the low rates of detection, as could inadequate methods of analysis. The pathogens can survive for long periods on and in these plant-based foods, and can propagate there under certain circumstances and potentially cause infection after consumption. As a result—and despite the comparatively rare rates of detection in the past—major outbreaks of foodborne illness have repeatedly been associated with contaminated fruit and vegetables. This is only logical, since these foods are often distributed over long distances, processed only minimally before consumption, and frequently consumed raw because of their healthy constituents by a majority of the population, including high-risk groups. In addition, these foods have only a short shelf life, which means that they have typically been consumed well before the outbreaks can be identified and traced.

Fresh fruit and vegetables can become contaminated with human pathogens during cultivation and harvesting, during transportation and processing, and during packing and storage. In open-air cultivation, plants can become contaminated from contact with soil, via irrigation water, agricultural practices, and by wild animals. Important sources of input into the soil include animal excreta present in organic fertilisers and from wild animals, contaminated surface water and water used for irrigation.

The microbiological condition of urban waste water depends on the infection status of the human population and of the livestock in the waste water catchment area. Urban waste water may contain a variety of bacteria, viruses and stages of parasites in varying concentrations, whose numbers are then reduced by the water treatment process depending on the suitability of the procedure. Concentrations of indicator bacteria can offer an insight into the success of this reduction process. One may assume that the occurrence of pathogenic gut bacteria in reclaimed waste water is directly proportional to the number of indicator bacteria detected in water samples. However, even if low concentrations of faecal coliforms and *E. coli* are found, one must still assume the presence of pathogenic bacteria, viruses and parasite stages. If contaminated waste water is used to irrigate food crops consumed raw because of its high

nutrient content and an increasing shortage in water supply, there is a risk that the pathogens will be transmitted directly or indirectly (via the soil) to the plant surfaces or actively invade the plants. The risk to health for the consumers of fresh fruit and vegetables could increase if pathogenic bacteria and bacteria with antimicrobial resistance propagate in biofilms that form in the downstream piping and hose lines of the irrigation system as a result of the higher concentration of nutrients. Such biofilms could also lead to temporarily high levels of microbes in irrigation water.

The persistence of human pathogens in agricultural soil cannot be estimated precisely, as a result of numerous biotic and abiotic influence factors in the soil, which are also themselves influenced to a varying extent by agricultural practices. Nonetheless, it is to be expected that *Salmonella*, STEC and *Listeria monocytogenes* will survive well—and likely propagate themselves—in moist parts of the soil enriched with nutrients by waste water. Strong solar radiation, however, is likely to have an antimicrobial effect in the upper layers of the soil.
The likelihood of an uptake of *Salmonella*, STEC and *Listeria monocytogenes* via the roots into the plant tissues, followed by internal migration, is rather low according to study results published to date, but may increase with high concentrations of the pathogens in the soil. This probability is further dependent on the characteristics of the bacterial strain in soil, the presence of competing microorganisms in the soil and the plant species.

If *Salmonella*, STEC or *Listeria monocytogenes* are—via the reclaimed waste water or by contaminated soil particles—transferred to plant surfaces, they may adhere to these for a period of time that also depends on the characteristics of the bacterial strain, the companion microbiota and the plant species. Strong solar radiation can also reduce bacterial populations on plant surfaces. In rare cases, *Salmonella* and STEC can invade plant tissues, especially the roots, by means of wounds or stomata.

There has so far been limited data on the distribution of *Salmonella* and STEC within plants. However, results of previous studies indicate that there is a greater likelihood of roots, leaves or parts of the plant near the soil containing these pathogens than fruits, for example. Regarding the distribution of *Listeria monocytogenes* within plants, no data that would permit an estimate of occurrence in individual plant parts have been made available to date. The only evidence to date is for a possible uptake into the roots.

A variety of scenarios are introduced in the following sections to estimate the impact of various irrigation systems on the potential occurrence of *Salmonella*, STEC and *Listeria monocytogenes* on and in fruit and vegetables intended for human consumption to be eaten raw, if plants are watered with reclaimed waste water during cultivation.

Scenario 1: During the cultivation of fruit and vegetables for raw consumption, the plants are irrigated using below-ground drip irrigation (or under sheeting, using plastic mulch) with reclaimed waste water:

> It is possible that *Salmonella*, STEC or *Listeria monocytogenes* may enter deeper soil layers via the reclaimed waste water. Presumably, the bacteria can then survive at low levels for some time there, especially in the vicinity of the drip points. More precise predictions concerning persistence in deeper soil layers are not possible, however, due to a lack of data. The likelihood of an uptake of *Salmonella*, STEC and *Listeria monocytogenes* into plants via the roots depends on a large number of factors. While this uptake can be estimated as low based on research to date, it does appear to increase with the quantity of pathogens present in the soil.
> One may therefore always assume a risk of contamination or colonisation of the below-ground plant parts that are intended for (raw) consumption (such as root crops). The

transmission of human pathogens followed by the external colonisation of edible above-ground parts of the plant as a result of a direct contact between the roots and the reclaimed waste water or via contaminated soil particles or by an uptake via the roots and translocation into above-ground plant parts is, however, very unlikely in the case of below-ground drip irrigation.

Scenario 2: During the cultivation of fruit and vegetables for raw consumption, the plants are irrigated using above-ground drip irrigation with reclaimed waste water:

It is possible that *Salmonella*, STEC or *Listeria monocytogenes* may enter locally the upper soil layers via the reclaimed waste water. It is to be assumed that the bacteria survive for many weeks at the moist and nutrient-rich drip points, and may even propagate if conditions are favourable. A colonisation of the roots is very likely in this scenario. In rare cases, human pathogens can invade plants via the roots. The rest of the soil surface remains dry, which makes it harder for any bacteria present to survive.
During heavy rainfall or harvesting, root crops in particular are at risk of having human pathogens transmitted along with soil particles onto edible parts of the plant, where they adhere and may also invade the plant tissue. Since human pathogens are expected to survive only in the immediate vicinity of the drip points, however, transmission is here less likely than with other kinds of above-ground irrigation methods.
The likelihood of a direct transmission of any pathogens present in the reclaimed waste water to the plants is considered to be very low with this irrigation method.

Scenario 3: During the cultivation of fruit and vegetables for raw consumption, the plants are irrigated with reclaimed waste water using irrigation ditches:

It is possible that *Salmonella*, STEC or *Listeria monocytogenes* get extensively into the upper soil layers via the reclaimed waste water. It is to be assumed that the bacteria survive for many weeks in the moist and nutrient-rich ditches, and may even propagate if conditions are favourable. A colonisation of the roots is very likely in this scenario. In rare cases, human pathogens can invade plants via the roots.
During heavy rainfall (due to rainwater run-off) or harvesting, root crops in particular are at risk of having human pathogens transmitted along with soil particles onto edible parts of the plant, where they adhere and may also invade plant tissue.
With fruit and vegetables that grow near to the ground, external colonisation of the plants by direct contact with the reclaimed waste water is also possible. This is less likely than with sprinkler systems, however.

Scenario 4: During the cultivation of fruit and vegetables for raw consumption, the plants are irrigated with reclaimed waste water using sprinkler systems:

It is possible that *Salmonella*, STEC or *Listeria monocytogenes* get extensively into the upper soil layers via the reclaimed waste water, where they survive for many weeks, may even propagate in the moist parts of the soil, and—in rare cases—could invade plants via the roots.
External colonisation is also possible as a result of direct contact with the irrigation water. Fruit and vegetables growing near to the ground are at risk of having human pathogens transmitted along with soil particles onto edible above-ground parts of the plant, where they adhere and may also invade the plants.

Scenario 5: Fruit and vegetables for raw consumption are cultivated hydroponically with reclaimed waste water:

It is possible that *Salmonella*, STEC or *Listeria monocytogenes* present in the reclaimed waste water invade plants via the roots. According to research conducted to

date, the likelihood for plant colonisation is higher than with cultivation in soil, presumably because in the hydroponic system the pathogens are not bound to soil particles and the competing microorganisms present in soil are absent.
During the harvest, a transmission of the human pathogens onto edible parts of the plant followed by external colonisation is also possible as a result of direct contact with irrigation water.

The risk to consumers of falling ill following the consumption of fresh fruit and vegetables irrigated during cultivation with reclaimed waste water also depends on the plant species, the parts of the plant consumed (roots, stems, leaves, fruit), the preparation of the food and volume consumed (e.g. large quantities with lettuce but small quantities with kitchen herbs), the species and quantity of pathogenic bacteria ingested, and the consumer's personal sensitivity to these pathogens.

It is possible that the consumption of raw root crops and of fruit and vegetable species growing near to the soil presents a higher risk of infection—due to the greater probability of contamination as well as internal colonisation of the roots and external contamination with human pathogens—than the consumption of other fruit and vegetable species. The risk of infection appears even higher if plant roots are also eaten, as is the case with root crops (e.g. carrots) and sprouts. Peeling these vegetables can minimise the risk of a foodborne infection.

Since STEC are easily capable of surviving in and on plant-based foods and the infectious dose is very low, the risk of infection is not dependent on any further propagation within the food. In healthy adults, such infections would probably cause predominantly mild to severe diarrhoea. However, an infection can lead to haemolytic-uremic syndrome (HUS), especially in young children, which is associated with bloody diarrhoea and kidney failure, and which can lead to a long-term dependence on dialysis and even prove fatal in individual cases.

The probability of occurrence for cases of listeriosis would increase further if pathogens continue to multiply in these foods following harvest—by means of exudations of plant juices and inadequate refrigeration, for example. Nevertheless, it is unlikely that healthy adults in Germany would contract listeriosis following the consumption of fruit and vegetables irrigated with reclaimed waste water during cultivation. Certain groups of people are more at risk of contracting such an infection, however, as a result of having an undeveloped or weakened immune system. In these cases, the impairments to health are considered to be very severe and may frequently prove fatal. These groups of particularly sensitive individuals include the elderly, people with serious systemic disease (such as cancers or resulting from the long-term consumption of immunosuppressants), as well as pregnant women and newborns.

In healthy individuals, cases of gastrointestinal illness caused by *Salmonella* typically resolve fully within a few weeks. Severe cases of infection are rare. However, if *Salmonella* is capable of propagating, given moist and warm conditions on the plants or in the plant-based foods, this would not only increase the probability of occurrence for *Salmonella* infection, but could also mean that the infection progresses more severely. Infections may prove fatal in isolated cases, particularly in individuals of advanced age or with severe systemic disease.

Washing fruit and vegetables with clean drinking water is an appropriate way to reduce the concentration of bacteria adhering to the exterior. This method is not guaranteed to eliminate

all of the pathogens that may be present on and in the plants, however. Any pathogenic bacteria present in the plants would be inactivated only by heat, high-pressure treatment or irradiation.

3.1.4.1 Assessing the quality of the data

The BfR, the JKI and the MRI have prepared this health risk assessment concerning the potential for human infection following the consumption of fruit and vegetables irrigated with reclaimed waste water during cultivation on the basis of available data from the food control authorities of the German federal states, the scientific literature and the results of internal research activities. The quality of available data as well as the information related to the characteristics of *Salmonella*, STEC and *Listeria monocytogenes*, their behaviour in food, their transmission to humans and the illnesses triggered by these pathogens, can be assessed as satisfactory. The quality of data concerning the uptake of *Salmonella* and STEC into plants is also considered satisfactory. While data on the uptake of *Listeria monocytogenes* into plants are only sparsely available, they do permit estimates about probability. Data concerning the occurrence of pathogens in reclaimed waste water using different methods, and in soil and fresh fruit and vegetables, as well as the propagation and behaviour of pathogenic bacteria within plants are considered unsatisfactory.

3.1.4.2 Future research needs

Further research is needed to better assess the risk of human infection through the consumption of fresh fruit and vegetables irrigated with reclaimed waste water in the future. Reliable data need to be obtained on the occurrence and survival of human pathogens in reclaimed waste water, and on the efficiency of methods used to treat waste water in terms of the elimination or reduction of human pathogens. Further research is also needed on the mechanisms of uptake, behaviour and distribution of human pathogens in plants. The occurrence of VBNC cells from human pathogens on or in plants also presents a considerable challenge to diagnostics. Accordingly, research on reactivation and/or detection of these cell stages must be pursued.
There is also a need for research on the potential accumulation of human pathogens in soil, as well as on and in plants, as a result of repeated irrigation with reclaimed waste water. This research should consider the potential effects on patterns of uptake into plants and the compliance with hygienic-microbiological requirements. Indirect effects resulting from changes in the composition of the existing soil microbiota as a result of repeated irrigation should also be investigated, since the influence of soil microbiota on the survival and internalisation of pathogenic bacteria has been demonstrated.
Further research is also needed on the microbiome of crops that are cultivated hydroponically, especially in cases where reclaimed waste water is used, since it may offer increased potential for the establishment of human pathogenic bacteria as well as bacteria with transferable antimicrobial resistance.
Moreover, reliable data on the occurrence of human pathogenic bacteria and bacteria with transferable antimicrobial resistance of clinical relevance in plant-based foods in Germany are required. Ideally, these data should be broken down by country of origin for the foods concerned. An appraisal of foods from Asian and North African regions would be of particular interest in this context.
Consumption data for sensitive groups of individuals is also needed to estimate the significance of the consumption of fresh fruit and vegetables in terms of the frequency of listeriosis.

3.2 Other aspects

3.2.1 National and international requirements for irrigation water

The following section lists selected national and international standards, guidelines and recommendations that define requirements for the quality of irrigation water used for the cultivation of fruit and vegetables.

3.2.1.1 DIN 19650:1999-02 – Irrigation – Hygienic concerns of irrigation water

As with corresponding codes from other countries, the German DIN 19650 standard defines suitability classes according to the envisaged application, and defines hygienic-microbiological requirements for each suitability class. Irrigation water for outdoor and greenhouse crops intended for raw consumption may contain no more than 100 enterococci and 200 *E. coli* per 100 ml. Irrigation water with higher concentrations of indicator bacteria may be applied up to two weeks before harvest in the open-air cultivation of vegetables intended for raw consumption. *Salmonella* must not be detectable in 1,000 ml.

3.2.1.2 2017/C 163/01 – Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene

To provide assistance on the implementation of food law within the European Union, a guidance document was published on the use of good hygiene to address microbiological risks in fresh fruit and vegetables in primary production (2017/C 163/01). Concerning the irrigation of fresh fruit and vegetables that are likely to be consumed uncooked with reclaimed waste water, this document defines ‘treated waste water’ as waste water that has been treated to ensure that its quality is adequate for the envisaged use. In addition, the quality of the waste water must also meet the requirements specified in the national legislation of Member States or—if no such national legislation has been passed—the requirements specified in guidelines published by the World Health Organisation (WHO) on the safe usage of waste water and excreta in agriculture. Accordingly, the guidance document on the irrigation of fresh fruit and vegetables intended for raw consumption recommends a maximum level of 100 *E. coli* per 100 ml in reclaimed waste water, if it comes into direct contact with the edible parts of fresh fruit and vegetables. The maximum level is set as 1,000 *E. coli* per 100 ml if irrigation water does not come into direct contact with the edible parts of fresh fruit and vegetables intended for raw consumption. Section 7.3 of the guidance document also recommends taking into account the information provided by the following documents: ISO 16075-2:2015; the guidelines published by the WHO on the safe use of wastewater, excreta and greywater in agriculture and aquaculture (2006); and the guidelines published by the Food and Agriculture Organization of the United Nations (FAO) on irrigation water quality (1985).

3.2.1.3 ISO 16075-2:2015 – Guidelines for treated wastewater use for irrigation projects – Part 2: Development of the project

The proposed regulation dated 17 June 2019 cites ISO 16075 on multiple occasions. This standard can be applied in the following cases, for example:

- For the development of risk management plans
- For the systematic identification of hazards, as well as risk assessment and management
- As a basis for suitable additional barriers

Table 1 in ISO 16075-2:2015-08 defines the minimum requirements for the quality of reclaimed water intended for use in agricultural irrigation. The microbiological limits for thermotolerant coliforms are comparable with the requirements given in Annex I of the Council document. Unlike the Council document, however, ISO 16075-2:2015-08 recommends that, if no additional barrier is present, the irrigation of food crops intended to be consumed raw should always be done with the best category of reclaimed water (category A).

3.2.1.4 Guidelines published by the WHO on the safe use of wastewater, excreta and greywater in agriculture and aquaculture (2006)

The WHO guidelines specify maximum levels for the indicator microbe *E. coli* per 100 ml of reclaimed waste water for the various levels of waste water treatment in combination with different scenarios and health protection measures. The guidelines do not offer concrete microbiological criteria for specific suitability classes, however, as given in Guidance Document 2017/C 163/01 or DIN 19650.

3.2.1.5 FAO recommendations for irrigation water quality (1985)

The FAO recommendations do not specify microbiological criteria for irrigation water.

3.2.1.6 Guidelines for water reuse from the US Environmental Protection Agency (EPA) (2004)

Alongside a description of the hazards associated with water reuse and the various regulations of the different US states, the guidelines also include recommendations for specific limit values depending on the intended use of the reclaimed waste water. Accordingly, where reclaimed waste water is used for the irrigation of plants cultivated as crops that are intended for raw consumption, daily sampling should not, on average, detect any faecal coliforms in 100 ml of this water. In addition, no sample should exceed the limit value of 14 faecal coliforms per 100 ml. Where reclaimed waste water is used for irrigation of orchards, vineyards and in the cultivation of plant-based foods not intended for raw consumption, daily sampling should not, on average, detect more than 200 faecal coliforms in 100 ml and no sample should exceed 800 faecal coliforms per 100 ml.

3.2.2 Microbiological criteria in the EU for fresh fruits and vegetables and products thereof

Regulation (EC) No 2073/2005 (as amended) protects the health of consumers in the EU by specifying microbiological criteria for certain kinds of food. Compliance with these criteria is to be monitored by food business operators at certain process levels.
In accordance with the food safety criteria given in Annex I, Chapter 1, categories 1.2 and 1.3, ready-to-eat foods other than those intended for infants and for special medical purposes must not exceed a limit value of 100 CFU/g for products placed on the market during their shelf life. In addition, for ready-to-eat foods able to support the growth of *Listeria monocytogenes* (category 1.2), a complete absence of *Listeria monocytogenes* in 25 g must be demonstrated before the food has left the immediate control of the food business operator who has produced it, and in cases where the competent food authority is not satisfied that the limit value of 100 CFU/g can be maintained throughout the product's entire shelf life. Ready-to-eat foods with a shelf life of less than five days are automatically assigned to category 1.3 (ready-to-eat foods unable to support the growth of *Listeria monocytogenes*). Sprouted seeds are assigned to category 1.2, in accordance with Regulation (EU) 2019/229 amending Regulation (EC) No 2073/2005.

In addition, in accordance with the food safety criteria given in Annex I, Chapter 1, categories 1.18 to 1.20, the following ready-to-eat plant-based foods placed on the market may not contain any *Salmonella* during their shelf life in 5 x 25 g samples: sprouted seeds, pre-cut fruit and vegetables, and unpasteurised fruit and vegetable juices[9]. Certain STEC serotypes must also be undetectable in 5 x 25 g samples of sprouts (category 1.29).
For ready-to-eat pre-cut fruit and vegetables, and ready-to-eat unpasteurised fruit and vegetable juices[9], certain process hygiene criteria also apply according to Annex I, Chapter 2, categories 2.5.1 and 2.5.2 of this Regulation, with limit values specified for *E. coli*. If these limit values are exceeded, the food business operator must take steps to improve their production hygiene and review the sourcing of their raw materials.

4 Risk management options/measures

In contrast to DIN 19650 ('Hygienic concerns of irrigation water'), the microbiological criteria listed in Annex I of the present Proposal for a Regulation (dated 17 June 2019) do not stipulate any routine monitoring of the occurrence of *Salmonella* or of enterococci. The DIN also recommends further usage restrictions if the irrigation water contains more than 100 enterococci or 200 *E. coli* in 100 ml. The limit values listed for *E. coli* in Annex I of the present Proposal for a Regulation are nevertheless comparable with the recommendations made in Commission Notice 2017/C 163/01 and the requirements stipulated by ISO 16075-2:2015. However, they are considerably higher than the maximum limits for faecal coliforms in a guideline published by the US Environmental Protection Agency (EPA) (EPA, 2004) on water reuse, which are recommended to be applied for the daily monitoring of reclaimed waste water intended to be used for the irrigation of certain plants cultivated as crops intended for raw consumption.

The microbiological criteria specified in the current version of Regulation (EC) No 2073/2005 for the protection of consumers apply only to selected categories of food, and are not generally applicable to fresh fruit and vegetables. Accordingly, it is possible that contamination remains undetected, causing major salmonellosis or EHEC outbreaks, because fruit and vegetables can be marketed across very large regions, and are very frequently consumed raw by all sections of the population. Consequently, it is especially important to prevent the introduction of human pathogens into the food chain via reclaimed waste water.

Accordingly, the BfR, the JKI and the MRI recommend the following to protect against foodborne illness caused by pathogenic bacteria, viruses and parasites following the consumption of raw fresh fruit and vegetables:

1. For the hydroponic cultivation of food crops consumed raw, use only irrigation water of drinking water quality. This is because research findings indicate that pathogenic bacteria can easily colonise the roots and can then invade plants cultivated in this way via the roots. Pathogens must not be detectable in the irrigation water.
2. Restrict reclaimed water of quality category B to distribution using above-ground or below-ground drip irrigation. This is because these irrigation methods avoid direct contact with the edible part of the plant.
3. Restrict reclaimed waste water of quality category C to the irrigation of fruit trees, vineyards, feed plants and food products of plant origin that are not consumed raw.

[9] According to Regulation (EU) 2019/229 amending Regulation (EC) No. 2073/2005, ready-to-eat fruit and vegetables are exempted from these limits if they have been subjected to a bactericidal process whose effectiveness versus *E. coli* and *Salmonella* is comparable with that achieved by pasteurisation.

To protect against foodborne infections, consumers are also recommended to wash fresh fruit and vegetables thoroughly with drinking water before eating, to reduce the concentration of microbes present on the fruit/vegetable skin. Simply washing fruit and vegetables is not guaranteed to remove all of the pathogens that may be present, however. Consumers are therefore advised to peel or blanch vegetables that grow near the soil to reduce any risk of infection.
In addition, individuals with weak immune systems due to pregnancy, advanced age, pre-existing conditions or taking certain kinds of medicines are advised to protect themselves from foodborne infections by heating sprouts thoroughly before consumption. These groups of people are further advised not to consume pre-packaged, ready-to-eat salads. Instead, salads should be prepared just before eating from fresh ingredients that have been thoroughly washed.

Further information on the BfR website

Consumer advice: Protection against foodborne Listeria infections
https://www.bfr.bund.de/cm/350/verbrauchertipps-schutz-vor-lebensmittelinfektionen-mit-listerien.pdf

Consumer advice: Protection against infection with enterohaemorrhagic *Escherichia coli* (EHEC)
https://www.bfr.bund.de/cm/350/verbrauchertipps-schutz-vor-infektionen-mit-enterohaemorrhagischen-e-coli-ehec.pdf

BfR Opinion No 013/2019 of 12 April 2019: Resistant bacteria: Wash uncooked vegetables and lettuce thoroughly and prepare them fresh by yourself
https://www.bfr.bund.de/cm/349/resistant-bacteria-wash-uncooked-vegetables-and-lettuce-thoroughly-and-prepare-them-fresh-by-yourself.pdf

BfR "Opinions app"

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About the BfR

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany.

It advises the German federal government and German federal states ("Laender") on questions of food, chemical and product safety. The BfR conducts its own research on topics that are closely linked to its assessment tasks.

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