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# Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVHs). Part 1 (outbreak data analysis, literature review and stakeholder questionnaire)

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## Abstract

The contamination of water used in post-harvest handling and processing operations of fresh and frozen fruit, vegetables and herbs (ffFVHs) is a global concern. The most relevant microbial hazards associated with this water are: *Listeria monocytogenes*, *Salmonella* spp., human pathogenic *Escherichia coli* and enteric viruses, which have been linked to multiple outbreaks associated with ffFVHs in the European Union (EU). Contamination (i.e. the accumulation of microbiological hazards) of the process water during post-harvest handling and processing operations is affected by several factors including: the type and contamination of the FVHs being processed, duration of the operation and transfer of microorganisms from the product to the water and vice versa, etc. For food business operators (FBOp), it is important to maintain the microbiological quality of the process water to assure the safety of ffFVHs. Good manufacturing practices (GMP) and good hygienic practices (GHP) related to a water management plan and the implementation of a water management system are critical to maintain the microbiological quality of the process water. Identified hygienic practices include technical maintenance of infrastructure, training of staff and cooling of post-harvest process water. Intervention strategies (e.g. use of water disinfection treatments and water replenishment) have been suggested to maintain the microbiological quality of process water. Chlorine-based disinfectants and peroxyacetic acid have been reported as common water disinfection treatments. However, given current practices in the EU, evidence of their efficacy under industrial conditions is only available for chlorine-based disinfectants. The use of water disinfection treatments must be undertaken following an appropriate water management strategy including validation, operational monitoring and verification. During operational monitoring, real-time information on process parameters related to the process and product, as well as the water and water disinfection treatment(s) are necessary. More specific guidance for FBOp on the validation, operational monitoring and verification is needed.



**Keywords:** contamination, chlorine, fruit, herb, industrial setting, operational monitoring, peracetic acid, process water, processing operation, validation, vegetable, verification, wash water, washing, water disinfection treatment, water management, water quality, water replenishment, water safety

**Requestor:** EFSA

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**Waiver:** In accordance with Article 21 of the Decision of the Executive Director on Competing Interest Management a waiver was granted to an expert of the Working Group until 30 September 2021 and end of December 2021 respectively. Pursuant to Article 21(6) of the aforementioned Decision, the concerned expert was allowed to take part in the discussion and in the drafting phase of the EFSA Scientific Opinion on the 'microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVHs)', but was not allowed to take up the role of chairman, vice chairman or rapporteur of the Working Group within that timeframe. Any competing interests are recorded in the respective minutes of the meetings of the Working Group.

**Declarations of interest:** If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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## Summary

The European Food Safety authority (EFSA) asked the Panel on Biological Hazards (BIOHAZ) to provide a scientific opinion on the microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVHs) to provide guidance on the use of water in the production of ffFVHs, the establishment of microbiological requirements for water quality and the available prevention and control measures that can be implemented to maintain the appropriate microbiological quality of the water.

In particular the Panel was asked:

(1) to describe the microbiological hazards associated with the use of water in post-harvest handling and processing operations of ffFVHs and the routes and rates of contamination of the water and the ffFVHs; (2) to describe specific intervention strategies (i.e. water disinfection treatments, water replenishment, good hygiene practices, etc.) needed to ensure the appropriate microbiological quality requirements of water, used for post-harvest handling and processing operations of ffFVHs, taking into account their impact on the physiological state of the microbiological hazards present in the water; and (3) to describe relevant parameters to assess the appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of ffFVHs.

To address the mandate, a qualitative assessment was undertaken based on information retrieved from (i) a literature review including international reports, case-specific European legislation and scientific literature, (ii) an industry survey to gain insight on current industrial practices, (iii) data collection on food-borne outbreaks involving ffFVHs reported in the EU and non-EU countries (Norway, Switzerland and UK up to 2020) and (iv) zoonoses monitoring data in the EU.

Water used in post-harvest handling and processing operations accumulates organic matter and microorganisms originating from soil, plant exudates and debris. Process water has been identified as an important risk factor for the contamination of fruits, vegetables and herbs (FVHs). Special attention has been given to microbiological hazards associated with the use of contaminated water during harvest, post-harvest handling and processing, with a particular emphasis on cross-contamination during washing of ffFVHs. Among the different food industries, ffFVHs manufacturing industries, including packing houses and processing plants, are the most water-intensive due to the huge consumption of water to perform post-harvest handling and processing operations.

Summarised outbreak data are in line with previous EFSA opinions on food of non-animal origin (FoNAO), although there was evidence for an increased relative importance of some hazards, including *Listeria monocytogenes*, *Cryptosporidium parvum* and *Yersinia* spp. In particular, *L. monocytogenes*, *Salmonella* spp. and human pathogenic *Escherichia coli* can contaminate a wide range of FVHs, have a high impact on morbidity and mortality, and should, therefore, always be considered in the hazard analysis for ffFVHs. Leafy greens (fresh-whole or cut) were the main vegetable vehicle, associated with many hazards such as pathogenic *E. coli*, noroviruses, *Salmonella* spp., *L. monocytogenes*, *Y. enterocolitica* and *C. parvum*. Frozen FVHs, especially berries, were common vehicles for viral outbreaks. Additionally, frozen corn was the vehicle for a listeriosis outbreak. Other hazard and product combinations causing several outbreaks were sprouts and *Salmonella* spp. and kale and *C. parvum*.

There are three main sectors where handling and processing operations are relevant for water use. The three sectors are: fresh-whole FVHs, fresh-cut FVHs and frozen FVHs. Based on the industry survey and the literature review, most of the reported data focuses on washing of fresh-cut FVHs, followed by washing of fresh-whole fruits and vegetables.

Hazards are expected to contaminate FVHs during primary production, especially the zoonotic hazards transmitted via the faecal-oral route, e.g. *Salmonella* spp., Shiga toxin-producing *E. coli* (STEC), *Yersinia* spp. and *C. parvum*. However, hazard occurrence data along the production chain did not provide evidence for a specific point of contamination for any microbial hazard. Depending on the post-harvest operation, all hazards can potentially accumulate in the processing plant and lead to batch-to-batch cross-contamination.

The contamination rate of process water refers to the rate at which microbial contaminants are introduced into the process water over time during post-harvest handling and processing operations. It is typically expressed as the increase in microbial load per unit time. The contamination rate of process water depends on multiple variables, including the number of microorganisms in the contaminated product, the proportion of product entering the water that is contaminated, the ratio of product to water (w:v), the intervention strategies in place, as well as the transfer of microorganisms from product to water and vice versa from the water to the product.

A water management plan is based on two complementary pillars: (1) preventive measures, such as good hygienic and manufacturing practices, including technical maintenance, training of staff and cooling of post-harvest process water and, (2) interventions such as water disinfection treatments and water replenishment as water management strategies, which must be validated, monitored and verified under commercial operating conditions.

Interventions such as water disinfection treatments will be considered as 'efficacious' when these are able to maintain the microbiological quality of the process water to a level that avoids microbiological cross-contamination within the same batch and between different batches of ffFVHs during the handling and processing operations. According to the available literature, the studied water disinfection strategies include chemical-, physical-, biological- based treatments or combinations thereof. Their industrial-scale application is less studied than lab and pilot plant scale applications, noting that chemical-based water disinfection is more often investigated than physical and biological treatments. According to the EU industrial survey, chlorine-based disinfectants and peroxyacetic acid (PAA), either applied singly or combined with other disinfectants, are commonly used for treating process water used for processing (washing) fresh-whole and fresh-cut fruits and vegetables. These treatments have been shown to be able to avoid the increase of microbial load and even reduce it under lab-scale trials performed under optimum conditions. However, there is a dearth of information on their efficacy under industrial conditions for most of the processes and ffFVHs. Only chlorine-based biocides have demonstrated the capacity to avoid accumulation of microorganisms in process water under industrial conditions. However, their application should be properly managed to obtain the target result.

A good water management plan implies that any intervention (as a water management strategy) has to be validated, monitored and verified in their operation. The goal of the validation is obtaining evidence about the achievable microbiological quality of the process water to avoid cross-contamination during the handling and processing operations. Validation procedures allow FBOp to define the appropriate operational conditions associated with the water management strategy. Verification is regularly conducted as part of a Food Safety Management Systems (FSMS), to demonstrate that the applied water management strategies are working effectively and the process water reached the demanded microbiological quality (defined as fit-for-purpose for the intended use) to avoid cross-contamination of the ffFVHs via the water. The operational monitoring of the applied water management strategies aims at the follow-up of defined process parameters and conditions. Operational monitoring parameters should be selected from the evaluated factors in the validation study. To have an efficacious operational monitoring, real-time information of the parameters and conditions is necessary to be able to have timely corrective actions when one of the parameters or conditions is outside of its limits. The required real-time measurements must be done using calibrated devices, either off-line or with on/in/at-line methodologies.

The BIOHAZ Panel recommends that more information should be included in outbreak investigation reports, such as the origin of the raw FVH, and if it has been post-harvest processed as well as possible implications of different types of water as a source for the implicated pathogen. Also, specific and clear guidelines should be made available for FBOp to clarify the requirements on how water disinfection treatments can be used in the context of maintaining the microbiological quality of water used in the post-harvest handling and processing operations of ffFVHs. Moreover, technical guidance is needed on the procedures for the validation, operational monitoring and verification of the intervention strategies that can be applied as part of the process water management plan.

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# 1. Introduction

## 1.1. Background and Terms of Reference as provided by the requestor

There has been an increase in the number of reported outbreaks, cases, hospitalisations and deaths associated with food of non-animal origin (FoNAO) in the EU from 2008 to 2011 (EFSA BIOHAZ Panel, 2013). A tendency has been observed for the outbreaks associated with FoNAO to involve more cases, but be less severe than those associated with food of animal origin (Da Silva Felicio et al., 2015). Reports by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) show an increasing trend in the implication of foodstuffs of FoNAO on the total burden of food-borne outbreaks in Europe (Machado-Moreira et al., 2019). Moreover, frozen vegetables and fruit have been also associated with major outbreaks (Murray et al., 2017; Soon et al., 2020). There has been an increase in the number of reported outbreaks associated with fresh produce in Europe and North America in recent years (Aiyedun et al., 2021) as well as in the number of fresh and frozen berry-linked viral outbreaks globally (Bozkurt et al., 2020).

Potential sources of contamination of FoNAO attributed to primary production and processing operations have been reviewed by EFSA for various commodities including fresh and frozen fruit and vegetables (EFSA BIOHAZ Panel, 2011, 2013; 2014a,b,c,d,e, 2020). **Water use** during harvesting and processing has been identified as an important risk factor for contamination of fruits, vegetables and herbs (FVHs). Special attention has been given to microbiological hazards associated with the use of **contaminated water** during harvest, post-harvest handling and processing, with a special emphasis on cross-contamination during the washing of fresh and frozen fruits, vegetables and herbs (ffFVHs) (EFSA BIOHAZ Panel, 2014b). The process water used after blanching of vegetables in the deep-freezing industry is also important (EFSA BIOHAZ Panel, 2020). The microbiological quality of the water that comes into contact with ffFVHs is an important consideration and should be controlled by an operational prerequisite program (OPRP) activity to avoid cross-contamination (FAO and WHO, 2019; EFSA BIOHAZ Panel, 2020).

Large volumes of water are used during harvest and post-harvest handling and processing operations (e.g. washing, rinsing, fluming, chilling, cooling, and for general cleaning, sanitation and disinfection purposes), as well as during fresh-cut/freeze value-added operations, distribution and end-user handling of ffFVHs. Therefore, most post-harvest processors are in favour of using the same water during many hours of processing operations for sustainability reasons (i.e. to save water and energy) and because, in some regions, access to potable water is limited or very expensive. According to current practices, potable water is used to fill the equipment and tanks during the first hour in the morning and the water is not replaced for several hours or even several days in some cases, during which time large volumes of ffFVHs may be processed. Hence, organic matter, microorganisms, including pathogens, and chemical residues can accumulate in the water, causing cross-contamination between batches, and this is a major concern (FAO and WHO, 2019). The quality of water used in post-harvest handling practices as well as during processing operations of ffFVHs should be monitored and controlled to avoid accumulation of microbiological hazards.

Most current recommendations specify that post-harvest water that comes in contact with ffFVHs, and that is not usually subjected to an upstream microbiological inactivation or reduction treatment, should be of potable quality during all post-harvest handling operations (FAO and WHO, 2019).

According to Council Directive 98/83/EC 'water intended for human consumption'[1] shall mean among others 'all water used in any food-production undertaking for the manufacture, processing, preservation or marketing of products or substances intended for human consumption unless the national competent authorities (Cas) are satisfied that the quality of the water cannot affect the wholesomeness of the foodstuff in its finished form'.

Annex II – Chapter VII of Regulation (EC) No 852/2004 on the hygiene of foodstuffs[2] states that recycled water used in processing or as an ingredient is not to present a risk of contamination. It is to be of the same standard as potable water, unless the CA is satisfied that the quality of the water cannot affect the wholesomeness of the foodstuff in its finished form.

[1] Directive 98/83/EEC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.1998, p. 32–54.

[2] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1–23.

Additionally, paragraph 7.3.4.3.c in the EU Commission Notice (2017/C 163/01)[3] on guidance documents addressing microbiological risks in fresh fruits and vegetables (fFVs) at primary production through good hygiene indicates that, for primary production and associated operations at the place of such production (harvest and post-harvest), the washing water used should be at least of clean water quality for the initial washing stages. Water used for final rinses has to be of potable quality if the fFVs are often consumed as ready-to-eat (e.g. tomatoes, apples, pears, young carrots, spring onions).

According to paragraph 7.3.4.3.f in the EU Commission Notice (2017/C 163/01) as well as in relevant research papers (Gombas et al., 2017; FAO and WHO, 2019), if water is contaminated during washing and then used to process large quantities of ffFVHs, it can be a vehicle for cross-contamination.

To avoid cross-contamination of the product due to the use of contaminated water, water disinfection treatments are needed to eliminate, or reduce to an acceptable level, microorganisms of public health concern but these treatments should not have an adverse effect on the quality and safety of the produce. Therefore, regardless of wash method used, growers and processors should follow good practices that ensure and maintain an appropriate water quality.

National rules within Member States exist and may create trade barriers since some prohibit any use of water disinfection treatments in process water, while such practice is common in others. These risk management decisions are often based on different considerations about the reduced risk associated with microbiological contamination versus the potential added chemical risk associated with their use.

Moreover, concerns may arise regarding maintenance of the microbiological quality of process water as well as the application of water disinfection treatments by the food business operators (FBOp). The proper operation of water disinfection treatment (e.g. application rate, in-use concentration and residual concentration on ffFVHs) as well as of the monitoring of the efficacy has to be conducted in a proper and safe way. As established by FAO and WHO (2019), water quality must be maintained throughout the processing operation and special attention is required for common wash and flume systems and reused water.

Water quality and use in post-harvest handling and processing operations is an increasing concern at global level, mostly because there is an expected reduction in the availability of water of drinking quality due to climate change (CXC 53–2003).[4] During the 43th session of the Codex Alimentarius Commission on the Joint FAO/WHO Food Standards Programme in Autumn 2020, the future development of guidelines for the safe use and reuse of water in food production was approved. These guidelines will contain a specific Annex on the use and reuse of water in fresh produce production.

## Terms of Reference

The BIOHAZ Panel is asked to issue a scientific opinion on microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVHs) to provide guidance on the use of water in the production of ffFVHs, the establishment of microbiological requirements for water quality and the available prevention and control measures that can be implemented to maintain the appropriate microbiological quality of the water.

More specifically, EFSA is requested to address the following terms of reference (TORs):

**TOR1 to describe the microbiological hazards associated with the use of water in post-harvest handling and processing operations of ffFVHs and the routes and rates of contamination of the water and the ffFVHs.**

**TOR 1.1:** Which are the most relevant microbiological hazards associated with the use of water in different post-harvest handling and processing operations for ffFVHs?

**TOR 1.2:** What are the routes of water contamination and the rates of contamination (increase in microbiological and pathogen load over time) for the most relevant microbiological hazards (identified in TOR 1.1.) in the water used in different post-harvest handling and processing operations for ffFVHS?

**TOR 1.3:** Which are the contamination rates (increase in microbiological and pathogen load over time) for the most relevant microbiological hazards (identified in TOR 1.1.) between different ffFVHs batches during different post-harvest handling and processing operations using the same water?

[3] European Commission, 2017. Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (2017/C 163/01). OJ C 163, 23.5.2017, p. 1–40.

[4] CAC (Codex Alimentarius Commission), 2003. Code of Hygienic Practice for Fresh Fruits and Vegetables CXC 53–2003, p. 1–39.

**TOR2 to describe specific intervention strategies (i.e. water disinfection treatments, water replenishment rates, good hygiene practices, etc.) needed to ensure the appropriate microbiological quality requirements of water, used for post-harvest handling and processing operations of ffFVHs, taking into account their impact on the physiological state of the microbiological hazards present in the water.**

**ToR 2.1**: Which good hygiene practices are recommended to ensure appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of ffFVHs?

**TOR 2.2:** Which are the most efficacious water disinfection treatments (dose and mode of application) to maintain the appropriate microbiological quality requirements of water used during different post-harvest handling and processing operations of ffFVHs?

**TOR 2.3:** What is the impact of different water disinfection treatments on the induction of the viable but non-culturable (VBNC) state or injury state in bacteria in water used for different post-harvest handling and processing operations of ffFVHs?

**TOR 2.4:** Which are the relevant parameters to establish efficacious water replenishment rates needed to maintain the appropriate microbiological quality requirements of water used for different post-harvest handling and processing operations of ffFVHs?

**TOR3 to describe relevant parameters to assess the appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of ffFVHs.**

**TOR 3.1:** Which relevant parameters can be used to validate and/or verify the appropriate microbiological quality requirements of the water intended to be used for different post-harvest handling and processing operations of ffFVHs?

**TOR 3.2**: Which relevant parameters can be used to monitor the appropriate microbiological quality requirements of water that is being used during different post-harvest handling and processing operations for ffFVHs?

## 1.2. Interpretation of the Terms of Reference

The classification of FVHs in different food categories can be found in Appendix A. This classification has been based in a previous EFSA opinion (EFSA BIOHAZ Panel, 2013).

Ready-to-eat fresh and minimally processed fruits and vegetables are those fruit and vegetables intended for direct human consumption without any additional steps or action taken to reduce or eliminate microbial contamination (FAO and WHO, 2021b). Minimal processing may occur at harvest as well as at on farm post-harvest operations and in processing plants (EFSA BIOHAZ Panel, 2013). Minimally processed FVHs may include ready-to-eat (RTE) and non-RTE foods. Therefore, in this Scientific Opinion, **minimal processing of FVHs** is defined as any action applied to the initial product (e.g. cleaning, coring, peeling, chopping, slicing, washing, dewatering, packaging) and which is not included in the definition of processing from the Regulation (EC) No 852/2004[2]: 'processing means any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes'.

Frozen FVHs include both the whole products (not cut or shredded) and those that are divided into smaller portions. They can be single products or mixed products where different frozen FVHs are present (EFSA et al., 2018). In the industrial production process of frozen FVHs, washing might be conducted in different steps of the process. Some frozen FVH products are always subjected to blanching, some of them can be blanched or not, while some of them are never blanched (EFSA BIOHAZ Panel, 2020). Blanching is used to extend the shelf life of the frozen product as it inhibits further enzymatic activity during an extended shelf life in the freezer. However, some vegetables do not support the blanching process, mainly due to detrimental effects on the quality of the product. Traditionally, frozen FVHs are not RTE food and they are intended to be cooked before eating. However, the changing consumer behaviour towards 'healthy' and 'convenience' meals has changed consumer perception meaning that frozen vegetables are often considered by consumers as safe to eat without cooking. The Food Business Operators (FBOp) decides if the product is RTE or not. In case it is not RTE, the cooking instructions need to be validated as well as the storage/handling conditions (freezing, thawing, time/temperature conditions after thawing, etc.) (EFSA BIOHAZ Panel, 2020).

Within the remit of this opinion, fresh-whole, fresh-cut and frozen FVHs (including blanched and not blanched) are included.

Specifically, for **ToR1.1** and within the remit of this scientific opinion, the microbiological hazards include all microorganisms, which may adversely affect human health, and be present in foods along

the food supply chain where water might be involved, including bacteria, viruses and parasitic protozoa. Microbial toxins are excluded. In addition to the current known hazards, emerging microbiological hazards e.g. due to new production systems in primary production (reuse of agricultural/industrial water, aquaponics, urban agriculture, etc.), will be addressed. Further on opportunistic pathogens are defined as those microorganisms that usually do not cause disease in healthy people but may cause disease in immunocompromised and unhealthy individuals. In the past decades, several microorganisms normally occurring in foods have emerged as opportunistic pathogens in humans and animals (Fusco et al., 2018).

For **ToR 1.2**, **water contamination routes** include both the potential contamination coming from the water source as well as the contamination coming from the FVHs and the environment of the post-harvest handling and processing operations, including FVHs, staff and equipment, among others. In this scientific opinion, the term '**process water**' is used as a synonym of the concept of '**fit-for-purpose**' water, to encompass all types of water that can be used in different post-harvest handling and processing operations including **clean water**, **recycled water** or **recirculated water,** knowing that the specific characteristics of process water should be adapted to the specific context and intended use. All these terminologies are described in the glossary of this opinion.

The **contamination rate** is defined as the change of the microbial load in **process water and ffFVHs** per time unit (usually the increase over a given time) during the processing operation, mainly because of the amounts of contaminated product entering the specific operation where water is used, and the survival of cells against exposure to the available free chlorine.

Particularly for **ToR 2**, the **appropriate microbiological quality requirements of water** refer to the fit-for-purpose concept and takes into consideration the context of safe water used in processing as defined by FAO and WHO (2019). The requirements for water quality used along the food chain must be considered within a fit-for-purpose concept, where the purpose of the water's use, potential hazards associated with the water use and whether there is any subsequent measure to decrease the potential for contamination further along the food chain are considered. For **ToR 2.1,** it is clarified that good hygiene practices (GHPs) are not considered as intervention strategies aiming to reduce the microbial loads but as prevention of microbiological contamination. However, within the remit of this scientific opinion, it is considered that any intervention strategy requires that all prevention strategies are first well-established and implemented.

For **ToR 2.2** the term water disinfection treatments will be used to describe the different treatments including biocides and physical disinfection treatments used to maintain the microbiological quality of the process water with the purpose of avoiding cross-contamination of ffFVHs. The definitions for biocides, sanitiser, disinfectant, water disinfection treatment and efficacy are included in the glossary of this scientific opinion. Within **ToR 2.2** some topics are out of the scope of this opinion, including:

- the link between the impact of water disinfection treatments on the reduction of microorganisms in process water and the contamination of ffFVHs washed with this water;
- the public health risk associated with the consumption of the ffFVHs processed with this water; and
- the evaluation of the efficiency of a treatment, which includes a cost–benefit analysis.

For **ToR 3**, both microbiological and physico-chemical parameters of the process water will be considered to assess the appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of ffFVHs. The analytical methodologies used for each specific parameter may include both direct and indirect measurements.

Within the remit of this opinion, the terms of validation, verification and operational monitoring will be used as part of the process water management system in post-harvest handling and processing operations of ffFVHs. The definitions of these terms are included in Section 3.12 as well as in the glossary.

For **ToR 3.1** and **ToR 3.2** real time operational monitoring can be performed on-line, in-line, at-line and off-line. The definitions of these terms are based on the distance between the ffFVHs processing line and where the measurement takes place.

## 1.3. Additional information

### 1.3.1. Approach to answer ToRs

The approach to answer the ToRs was defined in advance and is described in the protocol as well as the modifications that needed to be implemented during the assessment stage (Annex A). It covers both the problem formulation (i.e. what the assessment aims to address) and which methods will be used for addressing the problem. The problem formulation ('what') includes the clarification of the mandate (see further refined in Section 1.2) and consists of the steps (1) translation of the mandate into scientifically answerable assessment questions (AQs), (2) definition of the sub-questions (SQs) of each AQ and their relationship (conceptual model) and (3) the selection of the approach for the assessment. The planning of the methods for conducting the assessment ('how') consists of (1) specifying the evidence needs and the methods for answering each AQ or SQ, including uncertainty analysis and (2) the methods for integrating evidence across SQ and addressing of the remaining and overall uncertainty. Protocol development followed the draft framework for protocol development for EFSA's scientific assessments (EFSA, 2020). The framework is a draft because it will be refined and published after the trial phase over a year.

The AQs and SQs can be found below; their relationship can be found in the protocol and the conceptual model is shown in Figure 1.

- **AQ1.** Which are the microbiological hazards most relevant for public health in the EU that are associated with the use of water in different post-harvest handling and processing operations for ffFVHs?
  - **SQ1.** Which are the relevant combinations of ffFVHS /handling and processing operations requiring the use of water?
  - **SQ2.** Which are the most relevant microbiological hazards associated with the previously identified combinations of ffFVHS/handling and processing operations requiring the use of water?
  - **SQ3.** Which are potential emerging microbiological hazards due to changes in agriculture practices in cultivating ffFVHs?
  - **SQ4.** Which are the potential waterborne (including opportunistic) hazards associated with water sources used in the handling and processing operations of ffFVHs?
- **AQ2.** What are the routes of contamination for the most relevant microbiological hazards (as identified in AQ 1) in the water used in different post-harvest handling and processing operations for ffFVHS?
- **AQ3.** What are the rates of contamination for the most relevant microbiological hazards (as identified in AQ 1) in the water used in different post-harvest handling and processing operations for ffFVHS and between different ffFVHs batches?
- **AQ 4.** Which good hygiene practices (GHPs) are recommended to ensure appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of ffFVHs? (Preventive measures).
- **AQ5.** Which are the most efficacious water disinfection treatments (dose and mode of application) to maintain the appropriate microbiological quality requirements of water used during different post-harvest handling and processing operations of ffFVHs?
  - **SQ 5.** Which are the most commonly applied disinfection treatments for water used during different post-harvest handling and processing operations of ffFVHs by the industry?
  - **SQ 6.** How do the physico-chemical parameters (organic matter (amount and composition), pH, conductivity, etc.) of the process water used during different post-harvest handling and processing operations of ffFVHs affect the efficacy of the most commonly used disinfection treatments identified in SQ5?
  - **SQ 7.** Among the commonly used disinfection treatments, which are the most efficacious to inactivate (to minimise prevent survival or persistence) of pathogenic and indigenous microorganisms (e.g. total plate count) in process water? (disinfection = Inactivation as log reduction)

- **AQ 6.** What is the impact of different water disinfection treatments on the induction of the viable but non-culturable (VBNC) state or injury state in bacteria in water used for different post-harvest handling and processing operations of ffFVHs?
  - **SQ 8.** What is the impact of the different water disinfection treatments on the physiological state of the most relevant microbiological hazards? SQ2 (for AQ1)/ What are the processing conditions (e.g. pressure, time, temperature) and packaging applied by industry?
  - **SQ 9.** Are VBNC bacterial cells able to recover and/or express virulence in vivo in ffFVHs after washing and during storage?
- **AQ 7.** Which are the most efficacious water replenishment rates (when applicable and/or in combination with disinfection treatments) needed to maintain the appropriate microbiological quality requirements of water used for different post-harvest handling and processing operations of ffFVHs?
- **AQ 8.** Which relevant protocols including parameters, analytical methods and frequency can be used to validate and/or verify the appropriate microbiological quality requirements of the water intended to be used for different post-harvest handling and processing operations of ffFVHs?
- **AQ 9.** Which relevant protocols including parameters and analytical methods can be used for real-time monitoring of the appropriate microbiological quality requirements of the water intended to be used for different post-harvest handling and processing operations of ffFVHs?

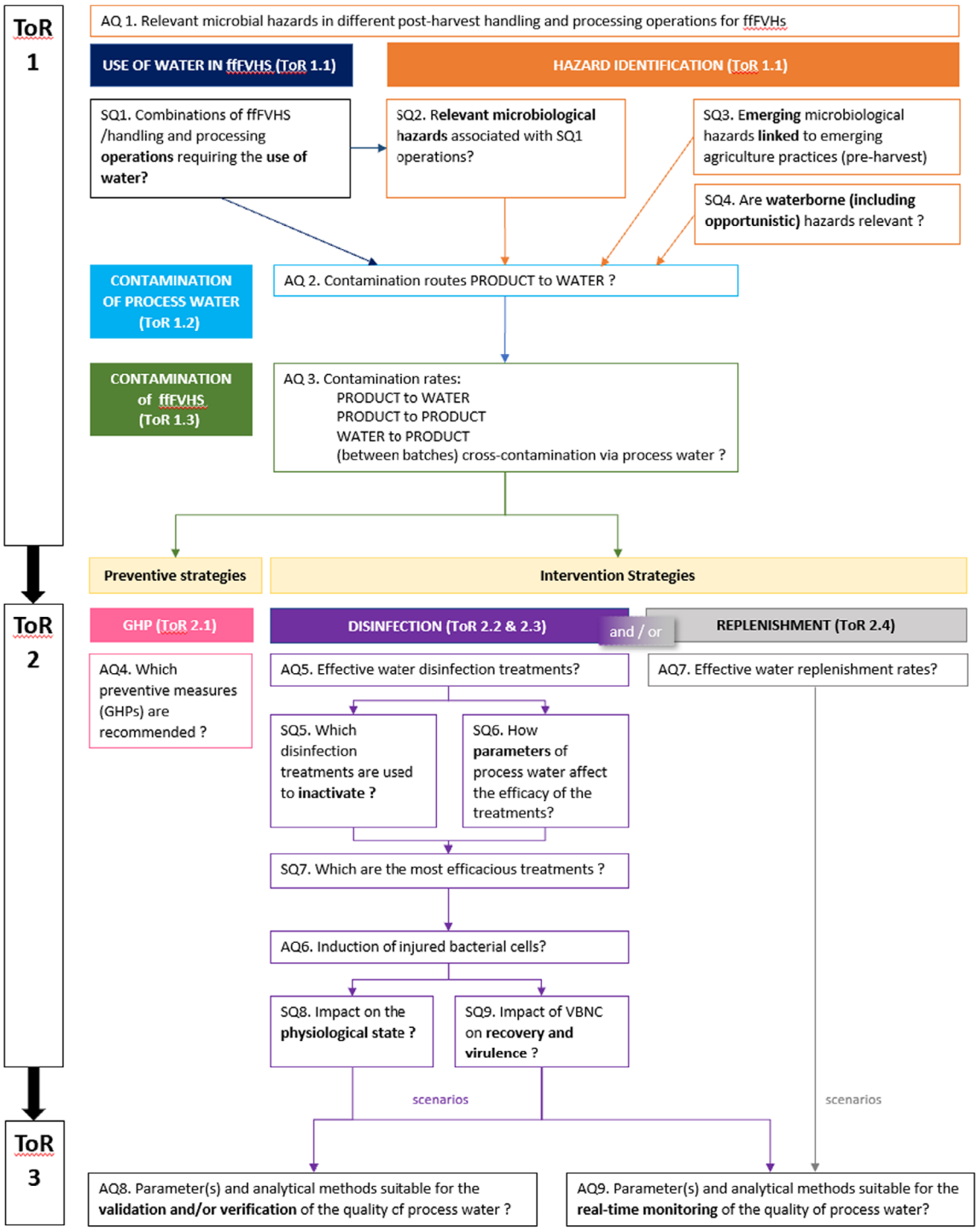



**Figure 1:** Conceptual model showing the relationships between the different AQs/SQs

# 2. Data and methodologies

## 2.1. Data collection

### 2.1.1. Literature search

The strategy for conducting the literature searches is provided in Appendix B. Searches were conducted in the following databases: Web of Science Core Collection (WoSCC) and Food Science and Technology Abstracts (FSTA), and CAB Abstracts (CABI). Only publications in English from 2010 until

the time of the search (15/2/2022) were included. Depending on the specific evidence needs, different filters were used, such as for the publication type (review articles, book chapters, articles, etc.), as well as inclusion and exclusion criteria (Appendix B). Further, information retrieved from other relevant sources (e.g. official documents, reports) generated by internationally recognised organisations (e.g. Codex Alimentarius, FAO/WHO, EU Commission) were considered, where appropriated. No national or industry guidelines were considered to avoid bias from specific countries or specific industrial practices and to avoid repetitive information (many of these are based on the international acknowledged organisations).

The records were screened in two steps: screening of (1) title and abstracts, and (2) full-text documents. For each of the literature searches, selected (on the basis of the assessment of the title and abstract) full-text documents were screened by one reviewer to extract the relevant information needed to answer the AQ or SQ. A systematic appraisal of the quality of each study was not performed, though the appropriateness of the methodological approach (including study design, methods, etc) was evaluated. Only when evaluating the efficacy of a technology/treatment, the strength of evidence of the different studies was considered to determine their potential extrapolation to the industrial conditions. The strength of evidence of those studies was assessed giving the highest relevance to those performed at industrial scale followed by pilot scale studies and lab scale studies.

The results of the literature searches are summarised in Table 1 which shows: (i) the number of records that have been retrieved for each AQ/SQ applying the search strategies described in Appendix B; and (ii) the number of records per AQ/SQ that were identified as relevant after 'title and abstract review' for full text review.

**Table 1:** Summary of literature searches

| Literature search number | AQ number or SQ number | Number of retrieved records | Number of records relevant after title and abstract screening (Excluding abstracts from proceedings and possible duplicates and including relevant references identified for other SQs/AQs) |
|---|---|---|---|
| 1 | SQ1 | 62 | 19 |
| 2a | SQ2 | 492 | 38 |
| 2b | SQ2 | 852 | 91 (based on title review only) |
| 3 | SQ3 | 87 | 41 |
| 4 | SQ4 | 294 | 54 |
| 2a | AQ2 | 492 | 71 |
| 2a | AQ3 | | 186<br>AQ3 SQ6 merged: 265 |
| | AQ4 | NA | NA<br>Only international guidelines |
| 5 | SQ5 | 584 | 53<br>AQ3 SQ6 merged: 265 |
| 5 | SQ6 | | 169<br>AQ3 SQ6 merged: 265 |
| | SQ7 | NA | NA |
| 6 | SQ8 | 436 | 34 |
| 6 | SQ9 | | |
| 7 | AQ7 | 21 | 7 |
| 8 | AQ8 | 124 | 58 |
| 8 | AQ9 | | |

NA: not applicable.

### 2.1.2. Food-borne outbreak monitoring data

Reporting of food-borne outbreak data is mandatory for the EU Member States, in compliance with Directive 2003/99/EC[5]. The current reporting system, known as the 'European Union Foodborne Reporting System' (EU-FORS), was implemented in 2010 and was updated in 2014 (EFSA, 2014). Outbreaks are categorised as having 'strong evidence' or 'weak evidence' based on the strength of evidence implicating a suspected food vehicle as the cause of the outbreak (EFSA, 2014).

Data on food-borne outbreaks implicating ffFHVs reported by EU and non-EU countries (Norway, Switzerland and UK[6]) to EFSA were used in the present Opinion to reply to ToR1, AQ1, SQ2. The aim was to retrieve information on the microbiological hazards associated with the ffFVHs. Only data on strong-evidence food-borne outbreaks reported from 2014 to 2020 and implicating the following three general food categories were included in the final data extraction: 'vegetables and juices and products thereof', 'fruits and juices and products thereof', and 'herbs and spices'. However, among the first two food categories, the following foods were excluded: all canned, cooked and dried products; vegetables or fruit juices (pasteurised or unpasteurised), fruits purees and dried fruits, coconut products, oil fruits and all unspecified categories for which a relevant food description was not provided. Salad and mixed salad were included in the data extraction, except those containing non-ffFHVs ingredients (e.g. RTE salad, salad with dressing, etc.) or if specified that they did not undergo any washing operation. Outbreaks implicating the consumption of sprouted seeds and minimally processed vegetables (e.g. pre-cut) were included in the data extraction. Pastes and dried products were excluded from 'herbs and spices'. During the food-borne outbreak data extraction process, the following food categories were preliminarily explored yet ultimately excluded because they did not include any foods relevant for this mandate: dates, multicomponent (composite) food products, 'other' foods, and 'cereal and cereal products'.

### 2.1.3. Zoonoses monitoring data

Reporting of monitoring data on zoonoses in animals, food and feed is mandatory for the EU Member States, in compliance with Directive 2003/99/EC[5]. According to List A of Annex I of Directive 2003/99/EC, data must be reported on a mandatory basis for the following eight zoonotic agents: *Salmonella*, *Campylobacter*, *L. monocytogenes*, Shiga toxin-producing *E. coli* (STEC), *Mycobacterium bovis*, *Brucella*, *Trichinella* and *Echinococcus*. In addition, and based on the epidemiological situations in the Member State, data must be reported on the following agents and zoonoses (List B of Annex I of the Zoonoses Directive): (i) viral zoonoses: calicivirus, hepatitis A virus, influenza virus, rabies, viruses transmitted by arthropods; (ii) bacterial zoonoses: borreliosis and agents thereof, botulism and agents thereof, leptospirosis and agents thereof, psittacosis and agents thereof, tuberculosis due to agents other than *M. bovis*, vibriosis and agents thereof, yersiniosis and agents thereof; (iii) parasitic zoonoses: anisakiasis and agents thereof, cryptosporidiosis and agents thereof, cysticercosis and agents thereof, toxoplasmosis and agents thereof; and (iv) other zoonoses and zoonotic agents such as *Francisella* and *Sarcocystis*. Furthermore, Member States can provide data on certain other microbiological hazards in foods: histamine, staphylococcal enterotoxins and *Cronobacter sakazakii*, for which food safety criteria are set down in the EU legislation (Regulation (EC) 2073/2005)[7].

In the present Opinion, the zoonoses monitoring data in food were used to complement the information provided by the food-borne outbreak data, to reply to ToR 1, AQ2. Only data from 2014 to 2020 on the occurrence of microbiological hazards in the following food matrices were extracted from the EFSA database: 'vegetables', 'fruits and vegetables', 'pre-cut fruits', 'vegetables (RTE)', 'unpasteurised fruit', 'vegetable juices (RTE)', 'fruits', 'mushrooms', 'coconut', 'spices and herbs, (fresh)', 'seeds, sprouted', 'seeds, sprouted – RTE', 'sprouted seeds (RTE)'. The products derived from the above-mentioned food categories (e.g. vegetable and/or fruit products, coconut products, etc.) were excluded from the extraction. All food matrixes were aggregated, and no distinction was made

[5] Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC OJ L 325, 12.12.2003, p. 31–40.

[6] The United Kingdom (of Great Britain and Northern Ireland) withdrew from the EU and became a third country on 1 February 2020 (Agreement on the withdrawal of the United Kingdom of Great Britain and Northern Ireland from the European Union and the European Atomic Energy Community. OJ L 29, 31.1.2020, p. 7). All of the data for the UK in 2020 were considered to be non-MS data.

[7] Regulation (EC) No 2073/2005 of the European Parliament and of the Council of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338,22.12.2005, p. 1–26 as amended by Commission Regulation (EC) No 2019/229 of 7 February 2019.

between batch and single sampling units, nor the type of sampler or sampling context. Only data from objective sampling was considered. The extracted data are summarised in unique tables showing the total number of tested units, the total number of positive units and the per cent positive per microbiological hazard (*Bacillus*, *Calicivirus*, *Campylobacter*, *Clostridium*, *Cronobacter*, *Cryptosporidium*, *Echinococcus*, *Giardia*, Hepatitis A virus, *L. monocytogenes*, *Salmonella*, *Shigella*, *Staphylococcus*, Shiga Toxin producing *E. coli* (STEC), *Toxoplasma*, *Vibrio* and *Yersinia*). The total number of tested units, the total number of positive units and the percentage of positive are presented for the following sampling stages: (i) farm, (ii) processing plant, (iii) packing centre, (iv) retail, (v) wholesale, (vi) mobile retailer or market/street vendor, (vii) catering, (viii) restaurant or cafe or pub or bar or hotel or catering service, (ix) school or kindergarten, (x) take-away or fast-food vendor and (xi) hospital or medical care facility (Appendix F). No occurrence data were reported in ffFVHs for *Bacillus*, *Cronobacter* and *Echinococcus* according to the above-mentioned selection criteria. Records with a sampling context reported as 'clinical investigations' and 'unspecified' were excluded, while all the other categories were aggregated.

#### 2.1.4. Industry survey

A survey with the purpose of increasing knowledge on current industrial practices on ffFVHs and the post-harvest handling and processing operations where water is used was sent on 19/9/2022 to the industries collaborating in the tender OC/EFSA/BIOCONTAM/2021/02[8]. No additional direct consultation of specific food industries or European industry associations was performed. The questionnaire was distributed by e-mail and included a pdf version of the questionnaire as well as a link to the on-line version.

The industry survey is provided in Annex B. It consists of (1) questions related to the details of each industry (characteristics of respondents), followed by (2) questions about post-harvest handling operations requiring the use of water as well as specific data on the type of water, the volume of water, ratio of product to water, etc. The questionnaire also comprised (3) questions related to any potential water disinfection treatments applied by the ffFVH processors, along with validation, verification and monitoring activities (including information about the parameters, analytical tests, etc.), as well as (4) current applied good hygiene practices to ensure appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of ffFVHs in European establishments. Finally, (5) questions on potential emerging cultivation techniques of ffFVHs as source of raw materials are requested, to get an insight in additional cultivation and potential sources of pathogens can be identified. The questionnaire was open between 19/9/2022 and 1/6/2023 (time period).

Information extracted from the received replies to the industry survey was analysed and summarised in the context of the assessments performed for SQ1, SQ3, SQ5, AQ8 and AQ9.

### 2.2. Microbiological hazards associated with the use of water in post-harvest handling and processing operations of ffFVHs and the routes and rates of contamination of water and the ffFVHs (AQ1)

#### 2.2.1. Overview of the post-harvest handling and processing operations (SQ1)

Two sources of information were used to provide an overview of relevant combinations of ffFVHs requiring the use of water post-harvest: (i) a literature search and (ii) the questionnaire to the industry. Data were extracted from these sources using summary tables, and the output is a list of the most important post-harvest handling and processing operations in relation to ffFVHs using water.

For the literature search, records were retrieved as described under Section 2.1.1. Studies were considered eligible when they fulfilled the following criteria: (i) reported any handling and processing operation where water is used in post-harvest activities for ffFVHs, (ii) dealt with different types of ffFVHs (fresh-whole, fresh-cut and frozen FVHs) and (iii) were conducted at the industrial or pilot scale. Review papers, official guidelines and book chapters were selected. When necessary, specific references included in the selected literature were consulted.

[8] https://etendering.ted.europa.eu/cft/cft-display.html?cftId=9577

### 2.2.2. Most relevant microbiological hazards associated with the use of water in different post-harvest handling and processing operations for ffFVHs (SQ2)

Two data sources were used to determine the most important microbial hazards associated with the relevant ffFVHs from SQ1: (i) a literature search and (ii) the food-borne outbreak monitoring data as described in Section 2.1.2.

For the literature search, studies were considered eligible when they fulfilled the following criteria: (i) described food-borne outbreaks of (ii) microbial hazards associated with combinations of ffFVHs for which handling and processing activities where water is used post-harvest (food type and food category). For this SQ, peer-reviewed papers (and official documents from other relevant sources as described in Section 2.1.1) were used.

For the food-borne outbreak monitoring data, data were extracted using summary tables and the output is a list of outbreaks, cases, hospitalisations and deaths from microbial hazards in relation to ffFVHs as outbreak vehicles.

### 2.2.3. Potential emerging microbiological hazards due to emerging agricultural practices (SQ3)

Two sources of information were used to determine potential emerging microbiological hazards due to emerging agricultural practices: (i) a literature search and (ii) the questionnaire to the industry.

For the literature search, records were retrieved as described in Section 2.1.1 and were screened for relevant information on potential emerging hazards due to new cultivation techniques used during ffFVHs primary production. In addition, the reference lists of these papers were further screened for additional relevant information. Studies from the initial screening were considered eligible when they fulfilled the following criteria: (i) described an emerging cultivation/ agricultural activity of ffFVHs where water is used post-harvest and (ii) described the food-borne pathogen that could be linked to ffFVHs cultivated by the emerging practices. Studies that described current, well established, agricultural practices: i.e. open field (soil) or greenhouse (soil or substrate cultivation), were excluded because this contamination route is covered by Section 2.2.2. For this SQ, peer-reviewed papers (and documents from other relevant sources as described in Section 2.1.2) were used.

### 2.2.4. Potential waterborne (opportunistic) hazards associated with the use of water in different post-harvest handling and processing operations for ffFVHs (SQ4)

One source of information was used to determine potential waterborne (opportunistic) hazards associated with the use of water in different post-harvest handling and processing operations for ffFVHs: (i) a literature search.

For the literature search, records were retrieved, as described in Section 2.1.1 and screened for relevant information on waterborne (opportunistic) human hazards that can multiply and/or form a biofilm during relevant conditions used for post-harvest handling and processing as identified in SQ1. The reference lists of these papers were further screened for additional relevant information. Studies were considered eligible when they fulfilled the following criteria: (i) described waterborne (opportunistic) hazards associated with ffFVHs handling and processing operations and biofilms, and (ii) were conducted at the industrial, pilot or lab scales. For this SQ, only peer-reviewed papers were selected. Based on discussion among the working group, the following criterion was applied to consider an organism as an 'emerging waterborne' hazard, namely when three or more papers reported the presence of the organism in the water applied in the post-harvest activities of ffFHVs. Moreover, the (opportunistic) hazards have as origin the water and the route for causing infection is the oral route.

Data were extracted using summary tables, and the output is the identification of the most important (opportunistic) waterborne hazards having the potential to proliferate and/or form biofilm during conditions used in relevant post-harvest processes identified in SQ1.

## 2.3. Routes of water contamination for the most relevant microbiological hazards associated with different post-harvest handling and processing operations for ffFVHS (AQ2)

Four sources of information were used to determine the routes of water contamination for the most relevant microbiological hazards associated with different post-harvest handling and processing operations for ffFVHs: (i) a literature search, (ii) EFSA zoonoses monitoring data, (iii) papers describing specific outbreaks and (iv) joint ECDC-EFSA rapid outbreak assessments (ROAs) in which the environmental investigation came to a conclusion on the most probable contamination route.

For the literature search, records were retrieved, as described in Section 2.1.1, to identify the relevant routes of water contamination. A study was considered eligible when it (i) included any post-harvest handling or processing activity where water is used, and any food-borne pathogen linked to ffFVHs; (ii) performed in industrial settings or during an outbreak investigation; and (iii) provided occurrence and/or quantitative data from different contamination sources to water.

Among the available data, only data on prioritised hazards from which more than 300 samples from at least two of the above stages were included in the analysis, (e.g. for *Salmonella* spp., *L. monocytogenes* and Shiga toxin-producing *E. coli* (STEC)). The threshold of 300 samples was selected as a reasonable number to avoid wide confidence intervals. Data were extracted using pre-defined tables where other relevant issues were collected, including the type of commodity, post-harvest activity and occurrence (prevalence and load) at three different stages (primary production, processing plant/packing house and retail).

## 2.4. Contamination rates for the most relevant microbiological hazards (identified in TOR 1.1.) in the water used in different post-harvest handling and processing operations for ffFVHs and between different ffFVHs batches (AQ3)

A literature search was used to retrieve information relevant to the contamination rates for the most relevant microbiological hazards and indicator microorganisms in the water used in different post-harvest handling and processing operations for ffFVHs and between different ffFVHs batches. For the literature search, records were retrieved, as described in Section 2.1.1, to collect quantitative data on the contamination rate over time from the FVHs to the water. The eligibility criteria for the literature review were: (i) the post-harvest handling and processing activity where water is used, and any food-borne pathogen or indicator microorganism that was linked to ffFVHs; and (ii) experiments were conducted at laboratory-scale, pilot-scale or industrial settings, on the condition that they provided usable data (e.g. quantifiable levels of pathogens in leafy greens and process water).

## 2.5. Preventive measurements: good hygiene practices (AQ4)

Based on a literature review, relevant good hygienic practices linked to postharvest water applications in the processing steps were identified. In this literature search, also guidance documents of acknowledged international and European organisations were screened for good hygienic practice advice and recommendations (see Section 2.1.2). Additional information on the currently applied good hygienic practices by the industry were further identified based on the industry survey (see Section 2.1.1) as a list of potential good hygienic practices was included in the industry survey (Annex B).

The information retrieved was assessed and classified based on the amount of information supplied (i.e. detailed, generic or absent) to have an overview of the hygienic practices and preventive measures in water management.

## 2.6. Water disinfection treatment (AQ5)

### 2.6.1. Most common disinfection treatments used to maintain the microbiological quality of process water (SQ5)

Two sources of information were used to determine the most common disinfection treatments used to maintain the microbiological quality of process water: (i) a literature search and (ii) the questionnaire to the industry.

For the literature search, records were retrieved, as described in Section 2.1.1 and screened for relevant information on such water disinfection treatments. The reference lists of these documents were further screened for additional relevant information. Studies were considered eligible when they fulfilled the following criteria: (i) describe any water disinfection treatments commonly used in different postharvest and handling processing operations by industry to maintain the microbiological quality of the process water, (ii) provide data on pathogen-specific $\log_{10}$ reductions/levels (e.g. challenge tests) in process water and/or the resistance of microbial indicators or indigenous microorganisms in process water when different disinfection treatments were applied to postharvest process water with different physico-chemical characteristics (e.g. concentration and composition of organic matter, conductivity, pH, temperature, etc.) or (iii) were conducted at pilot-scale or industrial settings. For this specific AQ6, peer-reviewed papers, book chapters and other relevant documents (e.g. guidelines and reports etc.) were selected. No geographical restrictions were applied to the studies.

### 2.6.2. Physico-chemical parameters of process water with an impact on the efficacy of the most used disinfection treatments (SQ6)

A literature search was carried out, as described in Section 2.1.1, to retrieve information on physico-chemical parameters of process water that have an impact on the efficacy of the most used disinfection treatments as determined in SQ5. Studies were considered eligible when they fulfilled the following criteria: (i) include data on the physico-chemical parameters of process water used during disinfection treatments; (ii) be conducted at a lab-scale, pilot-scale and/or at industrial settings and (iii) provide usable data, e.g. quantifiable levels of water quality related to pathogens or microbial indicators in leafy greens and wash water.

Data from eligible studies were extracted in a table. The data and tools comprised: (i) data describing the change of water quality over time; (ii) data describing the impact of factors on the efficacy of the water disinfection treatments; and (iii) mathematical models that describe the impact of the physico-chemical parameters on pathogen or microbial indicator levels in the process water. The relevant information, as detailed above, was obtained for the most important combinations specified in ToR2.

### 2.6.3. Identification of the most efficacious water disinfection treatments used to maintain the microbiological quality of process water (SQ7)

A literature search was carried out, as described in Section 2.1.1, to retrieve information on the efficacy of different water disinfection treatments used to maintain the microbiological quality of process water as determined in SQ7. Extracted data was used to compare different disinfection treatments using the absolute reductions or reductions of accumulation of microorganisms in process water between the control (untreated process water) and the specific water disinfection treatment. Seven sets of data were used to illustrate the efficacy of different biocides under different process conditions. These data sets were obtained from the quantitative data retrieved by the literature search and chosen due to the suitability of these studies to show differences among biocides and conditions applied during washing. Outputs from Sections 2.6.1, 2.6.2, 2.6.3 will be used to select the most efficacious water disinfection treatments.

### 2.6.4. Impact of different water disinfection treatments on the induction of the viable but non-culturable (VBNC) state (SQ8) and the ability of VBNC to recover and/or express virulence (SQ9)

To answer the two relevant questions, i.e. SQ 8 and 9, kinetic inactivation and growth data, as well as data characterising the physiological states of cells after exposure to disinfectant treatments were used. The records retrieved, as described in Section 2.1.2, were screened for relevant information on the physiological state of microorganisms as well as microbial resuscitation/recovery following exposure to disinfectants. The reference lists of these documents were further screened for additional relevant information. Studies were considered eligible when they fulfilled the following criteria: (i) microorganisms in process water having different characteristics, (ii) focused on water disinfection treatments identified in SQ5, (iii) report pathogen-specific data on the induction of VBNC (SQ8) or resuscitation and expression of virulent genes of VBNC cells present in fresh FVHs after washing and during storage (SQ9), (iv) be conducted at lab and pilot scale or in industrial settings. For these SQs, research papers as well as review papers and book chapters were selected.

### 2.6.5. Efficacious water replenishment rate to maintain the appropriate microbiological quality requirements of water (AQ7)

To answer AQ7, data collected from literature and questionnaires to industry were used. The records were retrieved as described in Section 2.1.2 for relevant information on different water replenishment strategies. The reference lists of these documents were further screened for additional relevant information. Studies were considered eligible when they fulfilled the following criteria: (i) reporting about process water parameters needed to determine water replenishment rates, (ii) providing data on the dilution factor, log reductions, (iii) be conducted at lab scale, pilot scale or in industrial settings. For this specific AQ, research papers, as well as review papers and book chapters were selected. Data were extracted using pre-defined tables reporting type of process water, volume of water, flow rate of the water, characteristics of the process water and corresponding reference.

## 2.7. Relevant protocols (including parameters, analytical methods and frequency) to validate and/or verify the appropriate microbiological quality requirements of the water intended to be used for different post-harvest handling and processing operations of ffFVHs (AQ8)

To answer AQ9, data collected from literature and questionnaires to industry were used. The records retrieved from the literature search, as described in Section 2.1.2, were screened to get information on (i) suitable parameters, (ii) (analytical) methods to determine said parameters as well as (iii) knowledge on the overall validation and verification procedures (design of experiments, frequency, sampling strategy (where and how), revision of the system, etc.). The latter could be used either in the studies to validate the efficacy of the water treatments or in the procedures to verify the (microbiological) quality of the water used in different post-harvest handling and processing operations for ffFVHs.

Studies were considered eligible when they fulfilled the following criteria: (i) reported any physico-chemical parameter, microbial indicator or a combination that correlate with the occurrence (prevalence and load) of relevant pathogen in the post-harvest water (included pathogens, based on output of AQ1); (ii) were conducted at industrial and/or pilot scales; and (iii) were coming from peer-reviewed original articles, reviews, reports, book chapters or guidelines. The strength of evidence of the different studies dealing with pilot studies were considered to determine their potential extrapolation to the industrial conditions.

## 2.8. Relevant protocols (including parameters, analytical methods and frequency) to monitor the appropriate microbiological quality requirements of the water intended to be used for different post-harvest handling and processing operations of ffFVHs (AQ9)

To answer AQ9, data collected from literature and questionnaires to industry were used. The records retrieved from the literature search as described in Section 2.1.2 were screened to get information on suitable parameters and their (analytical) methods in a real-time manner that could be used to monitor the microbiological quality of the water used in different post-harvest handling and processing operations for ffFVHs. These sources of information were also used to retrieve data about the associated critical limits to each parameter. Studies were considered eligible when they fulfilled the following criteria: (i) reported any physico-chemical parameter or a combination of parameters that correlate with the microbiological quality of the water or the performance of the water treatment; (ii) provide systems or methods based on at-line, on-line, in-line, off-line approaches enabling real-time measurements (ii) conducted at industrial and/or pilot scale (i.e. laboratory studies were excluded), and were conducted; and (iii) were coming from peer-reviewed original articles, reviews, reports, book chapters or guidelines. The strength of evidence of the different studies dealing with pilot studies were considered to determine their potential extrapolation to the industrial conditions. Relevant information was extracted using summary tables, and the output was an overview of potential protocols including parameters and analytical methods for real-time monitoring.

## 2.9. Uncertainty analysis

Experts identified uncertainties associated with the data, factors, models and parameters which can affect the outcome of the ToRs.

A list of uncertainty sources affecting the different AQs is shown in Appendix C.

In the case of the outbreak data, the strength of evidence is a qualitative measure of the uncertainty that a given food item is the true vehicle of the outbreak. Its assessment is based on multiple types of evidence linking the suspect food to exposure and illnesses (i.e. microbiological, epidemiological, descriptive, environmental, traceability (tracing back/forward) of the investigated foodstuffs). Although the data reporting rules follow the same standard EFSA harmonised specifications, there are differences in the sensitivity of surveillance systems and the type of pathogens under surveillance across the EU Member States and the non-EU reporting countries. This lack of harmonisation should be taken into consideration when analysing and interpreting the food-borne outbreak findings.

The overall uncertainty was assessed by incorporating the impact of (i) combined uncertainties and/or (ii) additional uncertainty sources.

Furthermore, regarding the information retrieved from the EU industry survey, the representativity of the respondents was assessed considering the number of countries and size of industries covered.

# 3. Assessment

## 3.1. Regulatory context in the EU on the use of water in post-harvest handling and processing of ffFVHs

The overview of relevant European legal and Commission Notice documents on use of water in postharvest activities for production of ffFVHS is given in Table 2. Potable water is defined in Directive 2020/2184/EC[9] and all MS must apply this directive from 12 January 2023 on.

**Table 2:** Overview of relevant European legal and Commission Notice documents on use of water in post-harvest activities for production of ffFVHS

| Topic covered | Document |
|---|---|
| Use of potable water or clean water as prerequisite in food safety management systems* | Regulation (EC) No 852/2004(a) of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs |
| Microbiological quality of postharvest water applied at primary production (farm – agricultural level) | Commission Notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (2017/C 163/01)(b) |
| Good Hygienic Practice in relation to water use in contact with food* | Commission Notice on the implementation of food safety management systems (FSMS) covering Good Hygiene Practices and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses (2022/C 355/01)(c) |
| Microbiological and chemical quality of potable water* | The Directive (EU) 2020/2184(d) of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption |

*: Not specifically addressing fresh fruits and vegetables, generic all foods.

(a): Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1–23.

(b): European Commission, 2017. Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (2017/C 163/01). OJ C 163, 23.5.2017, p. 1–40.

(c): European Commission, 2022. Commission notice on the implementation of food safety management systems covering Good Hygiene Practices and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses (2022/C 355/01). 16.9.2022, p. 1–58.

(d): Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1–23.

[9] Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast) (Text with EEA relevance) OJ L 435, 23.12.2020, p. 1–62.

For the **primary production** of FFVs, Annex I of Regulation (EC) No 852/2004[2] on the hygiene of foodstuffs is relevant: (Annex I — Part A — II.3 (a)) '…FBOps are to comply with appropriate Community and national legislative provisions relating to the control of hazards in primary production and associated operations, including measures to control contamination arising from air, soil, water, feed, fertilisers, veterinary medicinal products, plant protection products and biocides and the storage, handling and disposal of waste'. Also, (Annex I — Part A — II.5 (c)) 'FBOp producing or harvesting plant products are to take adequate measures, as appropriate, to use potable water or clean water, whenever necessary to prevent contamination'.

For FBOps not belonging to primary production, according to the current European legislation on the use of water in the **postharvest handling and processing of ffFVHs**, in principle, potable water needs to be applied when direct contact with foods may occur (Regulation (EC) No 852/2004). Furthermore, Annex II – Chapter VII of Regulation (EC) No 852/2004 on the hygiene of foodstuffs states that '**clean water'** (defined as 'clean seawater or fresh water of a similar quality') can be used in processing or as an ingredient if it does not represent a risk of contamination. In the hygiene legislation, they also refer to '**recycled water**' to be used in processing or as an ingredient, which is not to present a risk of contamination. It is to be of the same standard as potable water, unless the competent authority is satisfied that the quality of the water cannot affect the wholesomeness of the foodstuff in its finished form. This legislation is horizontal legislation, not specifically addressing ffFVHs processing.

This means that the use of clean, recycled or recirculated water is regulated by the competent authorities in the different EU MSs, which may define these terms differently. More specific and detailed information may be available from national guidelines and/or legislations, however, these have not been included in the literature study. The EU Commission Notice (2017/C163/01)[3] guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene, gives detailed recommendations on the quality of post-harvest water that can be used in contact with fruit and vegetables for FBOps in primary production.

In the EU we can differentiate two situations:

1) **FBOp belonging to the primary production** (farms, agricultural activities) are recommended to implement Good Agricultural Practices (GAP) and Good Hygienic Practices (GHP) and can follow the microbiological standards for their post-harvest water as formulated in the Annex II of the EU Commission Notice (2017/C163/01, Annex II, example of matrix to support microbiological risk assessment of agricultural water).[3] More specifically, a threshold of 100 colony forming unit (CFU)/ 100 mL *E. coli* was set for: (i) activities belonging to post-harvest cooling and post-harvest transport for non-RTE fresh fruit and vegetables, (ii) water used for first washing of products in the case of RTE FFVs and cleaning equipment and surfaces where the products are handled. It should be considered that for final washing and ice/water for cooling applied for RTE FFVs, water of potable quality needs to be applied. In this EU Commission Notice, following the 'fit-for-purpose' concept, the quality characteristics of the process water (clean water, recycled water and reused water) for a particular use, are defined based on specific contexts.

2) **FBOp not belonging to the primary production** (e.g. packing houses and processing industry facilities). In this case, it is up to the FBOp, active in the processing of ffFVHs, to develop and apply a food safety management system (FSMS) consisting of PRPs and a HACCP plan based on a hazard analysis to identify potential CCPs or OPRPs related to the use of water, reuse of water and treatment of water during the production process of ffFVHs (included under the generic requirements of HACCP principles in Regulation (EC) No 852/2004)[2].

The monitoring and control of water quality is also covered in the current European guidelines. For instance, in the EU Commission Notice on the implementation of FSMS covering GHP and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses (2022/C 355/01)[10], a chapter is included about the water and air control, in which it is stated that 'Regular own microbiological and chemical analysis of water directly in contact with food (unless community potable water) should be carried out. Factors such as the source, intended use of the water, etc. will determine the frequency of the analysis'. Moreover: 'Control of water is an

[10] European Commission, 2022. Commission notice on the implementation of food safety management systems covering Good Hygiene Practices and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses (2022/C 355/01). 16.9.2022, p. 1–58.

important way of controlling microbiological and chemical hazards in the primary production of fruit and vegetables (e.g. washing at harvest)'.

As it is indicated, the European Commission's definition of 'clean water' is not the only one available. Different MSs, agencies and authorities define 'clean water' in different ways. In some cases, the definition of clean water is broader and allows more flexibility for producers and processors to be able to choose the most appropriate type of water depending on its use ('fit-for-purpose' water). For instance, the term 'clean water', defined by the Codex Alimentarius Commission (CAC) in CXC 53-2003 (CAC, 2003a) as 'water which does not compromise the safety of the food in the context of its use',[4] is used in a number of Codex texts. Based on this definition, the JEMRA meeting on the Safety and Quality of Water Used in Food Production and Processing (FAO and WHO, 2019), introduced the **'fit-for-purpose'** concept to give flexibility to the producers and FBOp to modify the requirements for water quality used along the food chain based on a specific context, considering the purpose of the water use, potential hazards associated with the water use and whether there is any subsequent measure to decrease the potential for contamination further along the food chain. Therefore, flexibility for producers and FBOp is granted, allowing them to use different types of water, if they can demonstrate that it does not cause contamination. This JEMRA report (FAO and WHO, 2019) also defined '**recirculated water'** as water reused in a closed loop for the same processing operation without replenishment (FAO and WHO, 2019). This is the case where the same volume of water is used for processing (large) volumes of product and is used for a given period from hours to weeks.

Therefore, depending on the specific definition of 'clean water' this can refer to a wider and more flexible concept or not, implying that it can be used as a synonym of 'fit-for-purpose water' or not. Nevertheless, the challenge for competent authorities or others implementing Codex standards and guidelines is how to translate this guidance recommending the use of 'clean water' or 'fit-for-purpose water' into operational guidance/targets for primary producers and food processors, allowing them to monitor such targets as part of their food control/food safety management programmes. The risk-based framework needed to define the 'fit-for-purpose water' requires both risk assessment and monitoring. In this opinion, it was decided to use the term **'process water'** as a synonym of the concept of '**fit-for-purpose water**', to encompass all types of water that can be used in different post-harvest handling and processing operations including **potable water**, **clean water**, **recycled water** or **recirculated water,** knowing that the specific characteristics of process water should be adapted to the specific context and intended use and should be risk-based.

#### 3.1.1. Main remarks

- Based on the currently available EU legislation different interpretations of clean water, recycled water and recirculated water are possible.
- This situation is leading to variability in the interpretation among different EU Member States about what is an acceptable CFU level of *E. coli*/100 mL in process water in contact with foods. However, within the EU, FBOp active in the handling and processing of ffFVHs (not primary production), need to include process water in the industry specific HACCP-analysis and the involved process steps need to be validated, monitored and verified.
- The Codex Alimentarius Commission (CAC) in CXC 53-2003 (CAC, 2003a) defines 'clean water' as 'water which does not compromise the safety of the food in the context of its use'.[14] This definition represents the basis for the introduction of the new 'fit-for-purpose' concept giving flexibility to the FBOp to modify the requirements for water quality used along the food chain (FAO and WHO, 2019). Within the 'fit-for-purpose' concept, potable water, clean water, recycled water and recirculated water of different types of qualities are included.
- In this opinion, the term 'process water' is used as a synonym of the concept of 'fit-for-purpose water', to encompass all types of water that can be used in different post-harvest handling and processing operations including potable water, clean water, recycled water or recirculated water, knowing that the specific characteristics of process water should be adapted to the specific context and intended use and should be risk-based.

### 3.2. Post-harvest handling and processing operations for ffFVHS requiring the use of water (SQ1)

In general, water is involved in many processing operations like washing, rinsing, fluming, chilling, cooling and general cleaning, sanitation and disinfection purposes, among others. The food industry is

characterised by high-water consumption per ton of food product (FAO and WHO, 2019). The main characteristics of water in the agri-food sector, and in particular in the post-harvest handling and processing of ffFVHs, are its diverse characteristics (e.g. microbiological quality and physico-chemical properties) as they vary not only across different ffFVHs (sector and facilities) but also within a particular food product (e.g. format) and handling or processing operation (Mundi et al., 2017). Among the different food industries, ffFVHs manufacturing industries, which include packinghouses and processing plants for ffFVHs (e.g. fresh-cut and freezing plants), are the most water-intensive due to the huge consumption of potable water to perform washing and freezing operations required to guarantee the safety and quality of the product (Manzocco et al., 2015; FAO and WHO, 2019). Most postharvest processors consider recycling of water (water, other than first-use water that has been obtained from a processing operation or water that is used in the same operation after reconditioning), as well as the use of recirculated water (water used in a closed loop for the same processing operation such as the use of the same water to wash/cool large volumes of FVHs) to save water and energy (e.g. for bin dumping, hydrocooling, flume recirculation and washing) (FAO and WHO, 2019). The diversity of handling and processing operations where water is applied makes a single, applicable approach to water uses and quality, complex.

The ffFVHs processing plants belong to three different sectors including: (a) fresh-whole, (b) fresh-cut and (c) frozen FVHs. Post-harvest handling and processing operations, applied in these three different sectors, requiring the use of water include:

a) **Postharvest process operations in the growing field**: After harvest, there are several activities that can occur in the field, such as cooling or dipping for de-coring (Figure 2). A key characteristic of these operations is that they involve considerable contact between FVHs and water or ice and the field environment, which may be contaminated with soil, dust and insects (Beuchat, 1996; Beuchat et al., 2001; Sapers et al., 2006). In most of the cases, fresh FVHs are hand-harvested into bags/boxes, transferred into field bins and transported to the specific facility (e.g. packaging, processing). In some cases, water bath bin dumps are used to reduce impact damage. The use of a water immersion dump is the preferred method to float fruits out of field bins. In ffFVHs, the harvest operations usually involve cutting edible parts from the plant, removing outer or damaged leaves, field coring, crating and film packing (Pradhan et al., 2018) (Figure 2). Antimicrobial or antioxidant solutions can be used to wash the cut surface but if aqueous solutions are reused, it is necessary to ensure that they do not become a source of contamination (Fallon et al., 2011; U.S. FDA, 2018).


**Figure 2:** Postharvest handling activities that can occur in the field, such as cooling or dipping for de-coring. © A. Allende

b) **Post-harvest handling operations out of the growing field:** Water is widely used in packing houses and processing plants for washing, fluming or cooling produce. Most of the commodities are routinely cooled immediately after harvest. Many different cooling systems

are used. For example, leafy greens are cooled down by spray-vacuum (or hydrovac) cooling and stone fruits by hydro-cooling. Transpiration or evaporation of water from the plant tissues, is one of the major causes of deterioration in fresh horticultural crops after harvest.

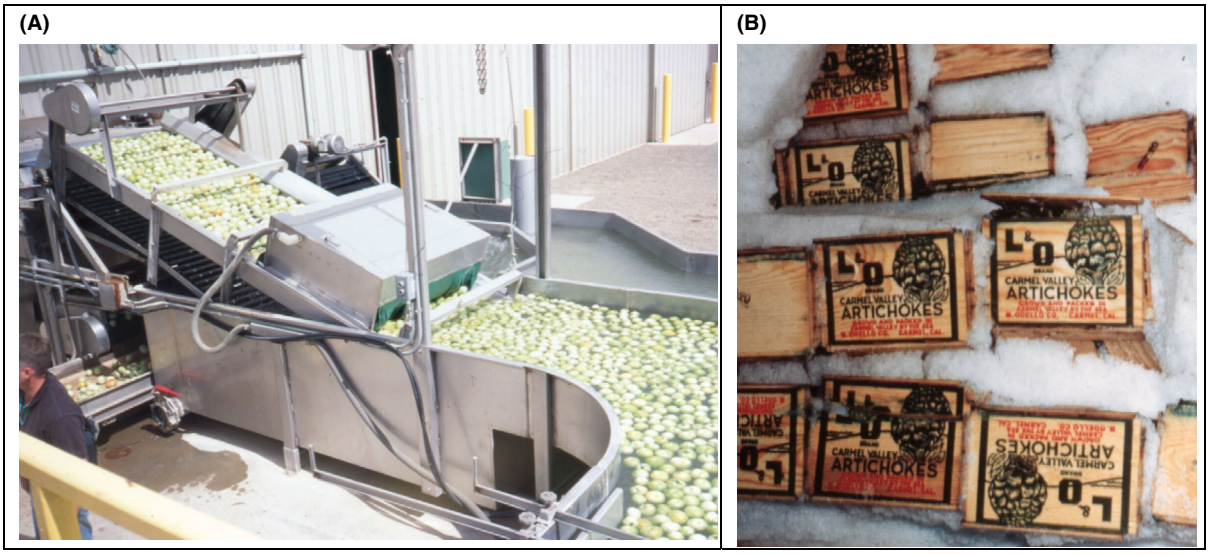



**Figure 3:** (A) Apple fluming and (B) Package icing. © M.I. Gil

Transpiration can be controlled through the direct application of water to the produce (surface coatings and other moisture barriers) or through manipulation of the environment (maintenance of high-relative humidity) (Martín-Belloso et al., 2012). Food-grade waxes, fungicides, calcium treatment and edible coatings may be applied to some fruits through a water immersion bath (Figure 3A). Package icing and liquid-icing are still used to a limited extent for cooling several commodities such as artichokes (Figure 3B). This cooling and shipping process with ice exposes the commodity to the water quality used to make the ice.

c) **Fresh-cut processing:** The fresh-cut industry is one of the food industries with higher water consumption and wastewater volumes in the range of 1.5–5 $m^3$/t and 11–23 $m^3$/t per finished product, respectively. Large facilities may require large volumes of water up to 100 $m^3$ of potable water per day for washing and processing needs. Washing is an important step in produce processing. After cutting, the cut product is immediately washed to remove soil, plant debris and exudates that occur during cutting and may support microbial growth (Figure 4). Most fresh-cut produce washing is conducted by immersing produce in tanks or flumes of process water, where water is used to wash large volumes of produce and which can be recycled to be used in other handling and processing operations (Gil et al., 2009). In commercial fresh-cut operations, wash system configurations vary greatly, including modifications such as open flume and closed flume systems and washing tanks (Luo, 2007). The fresh-cut products can be single-washed, double-washed or triple-washed, or various wash and spray combinations may be implemented. One option is the use of a 'triple-wash' procedure, where the cut produce is prewashed in a primary flume/tank (primary), followed by a wash in a second flume/tank with a residual concentration of a biocide (secondary) and finally by a clean water rinse to remove residual biocide from produce surfaces (Palma-Salgado et al., 2014). As Manzocco et al. (2015) described, 'almost 90% of water is used to perform washing operations, including primary washing to remove gross contamination, a number of consecutive immersions of the product in washing tanks and a final rinse step'.

**Figure 4:** Fresh-cut processing plant including the washing tanks and the flumes. © A. Allende

d) **Freezing plants:** In the frozen fruit and vegetable industry water is used in a similar way to that in the case of the fresh-cut processing industry. There is a high-water consumption for washing and blanching which generates large volumes of wastewater, like the water volumes used in the fresh-cut processing plants (Figure 5). In the fresh-cut processing plants usually two washing steps are included, while in the frozen industry, there is a washing step and blanching, whenever needed.

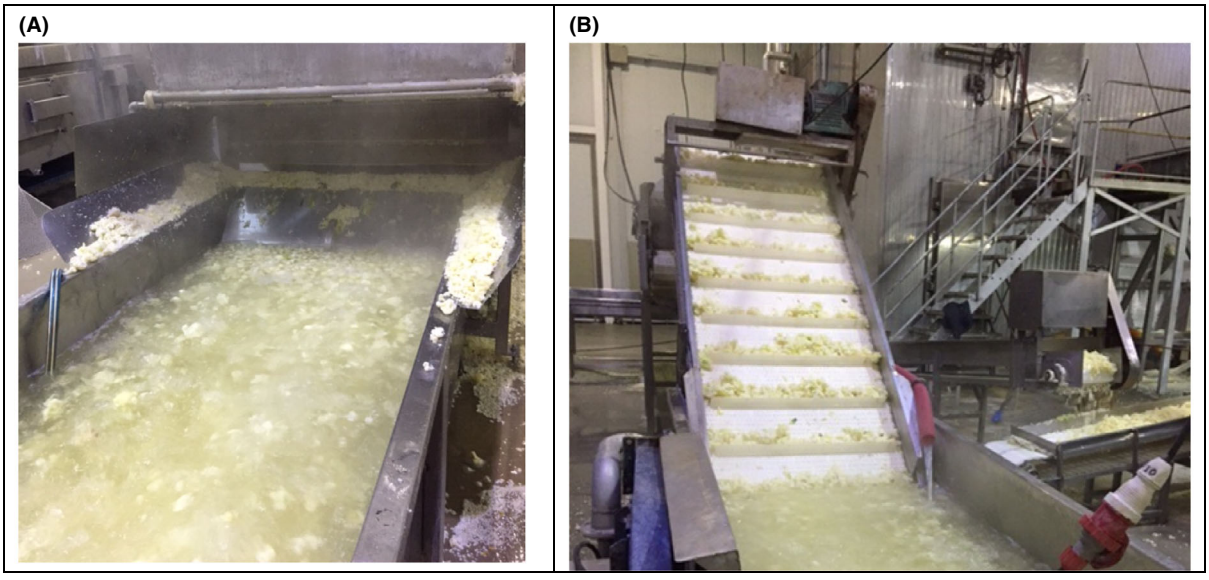



**Figure 5:** Cooling bath in a freezing plant. © A. Allende

From the EU industry survey, answers were obtained from a total of 31 industries, which reported information of 39 processing lines, including a total of 92 postharvest handling and processing operations. Among all the operations, respondents reported that washing is the most common post-harvest handling and processing operation for ffFVHs requiring the use of water. Washing, including pre-washing, was mentioned in about 55% (50/92) of the answers provided by the industry. Dumping is also a very common practice, included in about 19.5% (18/92) of the answers. The rest of the handling and processing operations reported included cooling and hydrocooling, blanching and rinsing, representing, each of them less than 5% of the replies. For instance, in 3/92 (3.3%) of all the operations, cooling after blanching was mentioned by 3/31 industries (9.7%). Some industries also reported the use of water for cleaning and disinfection operations of the processing lines, although this was out of the scope of this opinion. Information retrieved from the literature research was in line with the data retrieved from the questionnaire as most of the research papers focused on washing

operations (134/159 papers). A reduced number of papers focused on different handling and processing operations such as fluming or dumping, while even less covered on rinsing, hydrocooling and centrifuge water. Based on the results, the main post-harvest handling and processing operations for the three sectors (fresh-whole FVHs, fresh-cut FVHs and frozen FVHs) are: washing, rinsing, fluming and cooling.

#### 3.2.1. Main remarks

- Among the ffFVHs manufacturing industries, packinghouses and processing plants are the most water-intensive due to the huge consumption of water to perform post-harvest handling and processing operations.
- Post-harvest handling and processing operations requiring the use of water include: (1) post-harvest process operations in the growing field; (2) post-harvest handling operations out of the growing field; (3) fresh-cut processing operations; and (4) washing, blanching and cooling operations in the freezing processing plant.
- Based on the industry survey and scientific literature, washing is the most frequently reported and studied post-harvest handling and processing operation followed by dumping.
- Only few studies focused on rinsing, fluming, cooling/hydrocooling and centrifuge water. Although no scientific literature was found related to cooling after blanching, 9.7% of the industries reported this activity.

### 3.3. Microbiological hazards commonly associated with post-harvest handling and processing operations of ffFVHs as well as emerging microbiological hazards including waterborne human hazards potentially linked to process water (SQ2-SQ4)

#### 3.3.1. Outbreak data (SQ2)

A multi criteria analysis model aimed at risk ranking combinations of food of non-animal origin (FoNAO) and specific pathogens based on seven criteria was developed in EFSA BIOHAZ Panel (2013).[11] The top ranked combinations, using outbreak data from 2007 to 2011, were for the FHVs in question in the current opinion (i) *Salmonella* spp. and leafy greens eaten raw as salads; (ii) *Salmonella* spp. and bulb and stem vegetables; *Salmonella* spp. and tomatoes; *Salmonella* spp. and melons; and pathogenic *E. coli* and fresh pods; (iii) norovirus and leafy greens eaten raw as salads; *Salmonella* spp. and sprouted seeds; and *Shigella* spp. and fresh pods; (iv) norovirus and bulb and stem vegetables; norovirus and raspberries; *Salmonella* spp. and raspberries; *Salmonella* spp. and leafy greens mixed with other fresh FoNAO; *Shigella* spp. and fresh herbs, pathogenic *E. coli* and sprouted seeds; and *Yersinia* and carrots. The model was expected to overestimate the importance of some food/pathogen combinations, since only those reported in outbreaks in the EU as part of the Zoonoses monitoring were included and additional food/pathogen combinations may be identified as important if data from future EU monitoring is included. The model used was further likely to underestimate the importance of diseases which appear to be of a more sporadic nature, such as those due to *L. monocytogenes*, *Campylobacter* spp. and parasites (EFSA BIOHAZ Panel, 2013).

##### 3.3.1.1. EFSA outbreak data

The extracted dataset included information on 57 strong-evidence food-borne outbreaks associated with the consumption of ffFHVs reported from 2014 to 2020 by five EU Member States (Denmark, Finland, Germany, Italy and Sweden) and four non-EU countries (Norway, Serbia, Switzerland and the United Kingdom) (Appendix D). It should be considered that the use of this dataset has different limitations because for most of the outbreaks, no information has been reported indicating in which step of the food vehicle supply chain the food product contamination occurred (e.g. pre-harvest or post-harvest handling) and if water used in post-harvest handling and processing operations has been implicated.

Most of these outbreaks were caused by *Salmonella* and noroviruses (14 each), the latter frequently via contaminated frozen berries. Further, fresh berries and leafy greens have served as

[11] These criteria were: the strength of associations between food and pathogen, incidence of illness, burden of disease, dose–response relationship, consumption, prevalence of contamination and pathogen growth potential during shelf life.

vehicles for viral outbreaks (Appendix D). In addition to noroviruses two berry-related hepatitis A outbreaks were reported. *Salmonella* outbreaks were most often caused by vegetables, seven of them having sprouted beans or seeds as vehicles. Other ffFVHs implicated in *Salmonella* outbreaks were tomatoes, cucumbers, (pre-cut) zucchini, plums and (pre-cut) melon. Third in terms of number of outbreaks was *Cryptosporidium parvum* (6). Notably all these were reported from Sweden, four of them related to consumption of kale. Three *Shigella sonnei* outbreaks were reported, with fresh coriander, (imported) mint and snow peas as vehicles. Also, three *Yersinia enterocolitica* outbreaks were reported all related to leafy greens; spinach (2) and a salad mix. In terms of morbidity and mortality, 43 hospitalisations and 9 deaths were caused by *L. monocytogenes* infections after two outbreaks related to frozen corn and pre-cut leafy greens respectively. A total number of 107 hospitalisations and two deaths were further reported due to *E. coli* infections, most of them after consumption of leafy greens (Appendix D).

Of the 57 outbreaks, fruit, berries, juices and products thereof were the vehicle on 13 occasions. Eleven of these were viral outbreaks, nine norovirus and two HAV, on berries. The other two were related to *Salmonella* spp. from plums and pre-cut melons. For the 44 vegetable FBOs, the vehicles were leafy greens (21), sprouted seeds (6), vegetable fruits (6), herbs (5), legumes (2), sweet corn (2) and root and tuberous vegetables (carrot, leek, onions 2) (Appendix D).

#### 3.3.1.2. Outbreak data from literature review

The aim of the search was two-fold, firstly to gather data on combinations of FVHs and hazards on a wider geographical scale, although most non-European outbreaks were reported from the United States. Secondly, the search was done to get information on outbreak investigations to find evidence for contamination routes (see further Section 3.5). From the literature search, information from 87 outbreaks was gathered resulting in a total number of 17,833 cases, 604 hospitalisations and 17 deaths (Appendix E). Some of these are already included in the database mentioned above, however, sometimes with updated figures on number of cases after additional investigations including more extensive interviews and typing of patient samples (Sarvikivi et al., 2012; Müller et al., 2015, 2016; Rispens et al., 2020).

Thirty-six outbreaks were caused by frozen products, all but one due to the product hazard combination of berries and noroviruses. Fresh-cut products were implicated in nine outbreaks, all caused by leafy greens (eaten raw as salad) as a vehicle for *C. parvum*, *Y. enterocolitica*, *Salmonella* spp. and *L. monocytogenes* respectively. Most outbreaks were caused by fresh whole products (41), most often contaminated with *Salmonella* spp. but also different strains of pathogenic *E. coli*, *Campylobacter jejuni*, *S. sonnei*, noroviruses and *L. monocytogenes* (Appendix E). However, it was not always clear whether the fresh FVHs were fresh-whole or fresh-cut FVHs.

### 3.3.2. Emerging agricultural practices and associated microbiological hazards (SQ3)

Emerging agricultural practices refer to new or increased cultivation practices for raw materials such as fresh fruits, vegetables and herbs which may bring hazards into the food chain. The literature review on emerging preharvest cultivation techniques and potential microbiological hazards associated with such practices, suggests that different agriculture techniques based on **hydroponic systems, vertical farming and urban agriculture**, are being more and more implemented. Climate change signs (e.g. floodings, shortages of water) may be considered as a driving factor for the introduction of novel or emerging agricultural practices to overcome the negative impact on yields (Jacxsens et al., 2010).

**Hydroponics** is the technique of growing plants using a water-based nutrient solution rather than soil, and can include an aggregate substrate or growing media, such as vermiculite, coconut coir or perlite. The most commonly type of FVH commodities cultivated under these agricultural practices are tomato, lettuce or other leafy greens, including microgreens. Some studies suggest that substrates are a potential source of contamination in hydroponic systems which can facilitate microbial transfer to harvested leaves (Dankwa et al., 2020).

Hydroponics involve many different types of cultivation systems such as **deep-water culture hydroponics, aeroponics and aquaponics** (Figure 6). All are subsets of hydroponics. In aeroponics, the roots of the plants are suspended in the air. The way to provide water and nutrients to the plant is through a fine mist activated by a timer that sprays the roots to feed them and prevent them from getting dry. Indoor aquaponic systems use fish water as source of irrigation water to grow plants.

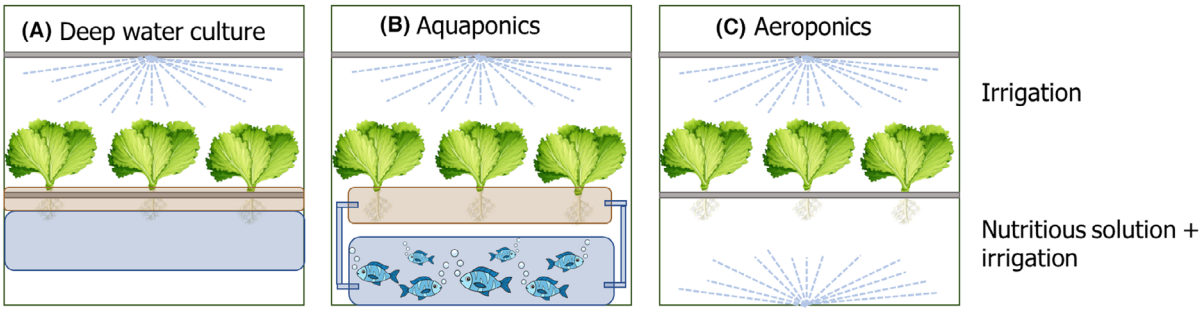



**Figure 6:** Common hydroponic systems: (A) deep water culture; (B) aquaponics and (C) aeroponics

In all hydroponic systems, the chance of food-borne pathogen introduction on fresh produce is usually lower compared to conventional soil-based agricultural systems. This is because of the lower likelihood of the edible part of the produce to come in contact with live animals or the manure thereof (Blidariu and Grozea, 2011; Tyson and Simonne, 2014; Kozai, 2016). Although the use of hydroponic growing systems reduces transfer of microbes from wildlife and soil, *Salmonella*, *E. coli* O157:H7, human noroviruses and *L. monocytogenes* have been identified on hydroponic produce (Lopez-Galvez et al., 2014; Shaw et al., 2016). EFSA BIOHAZ Panel (2011) has discussed the increased proliferation of inoculated STEC on microgreens when grown hydroponically, potentially due to a less competitive microflora. Highly controlled agricultural systems decrease the potential for a diverse and well-established bacterial community to develop. Leafy vegetables grown in highly controlled systems have both a 1–2 log lower total bacterial count and a lower microbiota diversity compared to their field-grown counterparts (Gomes Neto et al., 2012; Williams and Marco, 2014). This could decrease the ability of the microbiota to suppress introduced human pathogens. Moreover, the constant humid and warm conditions in the aquaponic system may favour the growth of pathogens (Turner et al., 2020).

Moreover, classical food-borne pathogenic microorganisms reported included STEC and *Salmonella* (Miceli and Settanni, 2019; Riggio et al., 2019; Wu et al., 2019; Buscaroli et al., 2021), among others like norovirus (Wang and Kniel, 2016; Riggio et al., 2019; Buscaroli et al., 2021), *Listeria* spp. (Miceli and Settanni, 2019; Riggio et al., 2019; Buscaroli et al., 2021) and some parasites like *Giardia* and *Cryptosporidium* (Buscaroli et al., 2021).

Regarding the type of water used within these emerging practices, more frequently, aquaponic fish water (Wu et al., 2019; Askari-Khorasgani and Pessarakli, 2020; Wongkiew et al., 2021) was applied, while in some other instances the use of recirculating systems (Bandi et al., 2016; Wang and Kniel, 2016) and clean water (Lee and Lee, 2015; Kyriacou et al., 2016) were reported. From the replies provided to the industry survey, one industry, mainly washing leafy vegetables, responded that hydroponics was involved for irrigation purposes during the production of their raw materials.

**Sprouted seeds** have different food safety concerns as compared to most other FVHs because the conditions under which they are produced (time, temperature, humidity, pH and nutrients) are ideal for food-borne pathogen growth. Outbreak investigations have demonstrated that food-borne pathogens found on sprouts most likely originate from the seed, but the contamination could also be attributed to the production environment (FAO and WHO, 2023). Sprouted seeds are vulnerable to pathogen survival or even proliferation (*L. monocytogenes, Salmonella* spp. and *E. coli* O157:H7) when seeds are contaminated, highlighting the importance of seed sanitation and proper water management during recycling water (Xiao et al., 2015; Reed et al., 2018; Wright and Holden, 2018; Turner et al., 2020).

**Shoots, cress and microgreens**. When a sprout grows its first leaves, it becomes a shoot if it is grown in water or a cress if it is grown in soil or substrate. The first leaves of a plant are called cotyledons and are different from the true leaves. Shoots are developed in water to produce a green shoot with very young leaves and/or embryonic leaves (cotyledons). Cress is usually sold as entire plants in substrate or soil (EFSA BIOHAZ Panel, 2011). Microgreens come after the shoots/cress stage. At the point of harvest, microgreens are anywhere from 2 to 4 weeks old. The average crop-time for fast-growing microgreens is 7–14 days but slower growing microgreens, may take 16–25 days. In all the cases (shoots, cress and microgreens) the growth stage is longer than the sprouted seeds and the seeds or roots are not kept in the final product. They are immature plants, which are highly perishable fresh produce, particularly vulnerable to microbial contamination, long-distance transportation and storage (Du et al., 2022). From the replies provided to the industry survey, one industry responded

that they wash young leaves, however it is not clear whether these could be characterised as microgreens.

**Vertical farms** are a novel type of farming in a controlled environment with a total replacement of solar radiation with artificial lighting that provides the necessary wavelengths of the spectrum for the growth and development of plants. In vertical farms, plants grow in hydroponic cultivation systems that allow stacking multiple layers or columns of plants horizontally or vertically. Vertical farms are in completely isolated spaces from outdoor environment with thermally insulated installations (especially when at the top floor of the building) and airtight structures that give the opportunity to the farmers to control the environment in terms of temperature, humidity and $CO_2$. There is also a 'low-to-no risk' of faecal contamination of crops by animals, a higher degree of control over water supply and reduced human handling of produce due to high extent of automatisation. However, these systems are still beholden to some of the same hazards as their lower tech counterparts as classical hydroponics – the microbial load of substrate, biofilms on surfaces, contamination of water used in irrigation, seed contamination, poor sanitation and poor processing and handling protocols (Avgoustaki and Xydis, 2020). From the replies provided to the industry survey, one industry responded that they bought FVHs from an external producer using vertical farming of vegetables and another industry responded that they would take part in a European project related to vertical farming and food safety.

FAO and RUAF (2022) have defined **urban and peri-urban agriculture (UPA**) as 'the production of food and other outputs and related processes, taking place on land and other spaces within cities and surrounding regions'. However, UPA has been defined in various ways in the literature, referring to a wide range of practices related to growing plants and/or raising animals for food and/or non-food purposes within and/or around cities. The emergence of the UPA concept, due to rapid urbanisation and increasing demand for local and sustainable food production, raises increased attention for research regarding UPA-related food safety risks (FAO and RUAF, 2022). Buscaroli et al. (2021) identified three factors as main food safety risk determinants related to water of UPA initiatives, being (i) soil vs. soil-less systems, (ii) the level of control, i.e. conditioned vs. non-conditioned systems and (iii) degree of circularity, e.g. the use of waste(water) or by-products in the framework of circular economy.

The use of **biopesticides** in fields as alternative to chemicals, such as microbial pesticides, which consist of a microorganism (e.g. a bacterium, fungus, virus or protozoan) as the active ingredient, can also be identified as an emerging agricultural practice as their use has increased in recent years. *B. thuringiensis (Bt)* is a biopesticide belonging to this category and one of the most often applied. *B. thuringiensis* biopesticide strains are under evaluation as a potential hazard to human health and the current use of *Bt* on edible plants raises concerns about food safety and public health (EFSA BIOHAZ Panel, 2016; De Bock et al., 2021; Zhao, 2023).

### 3.3.3. Emerging waterborne hazards linked to water applied in post-harvest production of ffFVHs (SQ4)

In this section we approached the literature search from the water perspective and not from the produce/food perspective. Literature related to the identification of emerging waterborne hazards present in various water sources which are applied in post-harvest handling and processing activities in the production of ffFVHs was screened. These hazards are currently not yet considered as a traditional food-borne hazard related to ffFVHs. However, due to their presence in the different water sources which are increasingly being applied by the ffFVH processing industry, they may become important in the future. As reviewed by Keuckelaere et al. (2015), risk assessment from a water perspective (and related water scientists) is not similar as from a food perspective. After the data extraction from the literature (44 papers, mainly papers form EU countries), a categorisation of different human (opportunistic) hazards was made based upon type of organisms (i.e. helminth, parasite, bacteria or virus) and the water source in which the pathogen was retrieved (surface water, well water, etc.). In the data extraction file, 159 records with bacteria, 3 records with helminths, 22 records for protozoa and 28 records with viruses were identified. Various papers are including similar species. These data were further classified according to species, type of associated water sources and number of papers which indicated that species. Based on discussion in the working group, the following criterion was applied to consider an organism as an 'emerging waterborne' hazard, namely when three or more papers (from different authors) reported the presence of the hazard in the water applied in the post-harvest activities of ffFHVs.

In total 28 different waterborne bacteria were identified, of which 21 are yet not identified as food-borne organisms in relation to ffFVHs (Sections 3.3.1 and 3.3.2). Based on the selection criteria used

to identify relevant waterborne hazards present in various water sources which are applied in post-harvest handling and processing activities in the production of ffFVHs, five emerging waterborne hazards can be listed: *Vibrio* spp., *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Clostridium perfringens* and *Arcobacter butzleri*.

In total 10 species of viruses were retrieved from the 44 papers of which only two are well-established (Norovirus and Hepatitis A virus) and two of these, namely Adenovirus and Rotavirus, were reported various times in the water-related papers. The other species are more sporadically reported.

Regarding helminths, only three species were identified in the 44 papers and each of them in another water source and only mentioned once. So, no conclusion can be made on the helminths from the water perspective.

In total six different protozoa were identified in the 44 papers. One, *Cryptosporidium* spp., has been well-established as a relevant hazard in the foods (Section 3.3.1). *Giardia* spp. can be identified as an emerging waterborne hazard in the production of ffFVHs. Others did not meet our definition of 'emerging waterborne' hazard, where three or more papers identified the species.

All other organisms are mentioned in a more fragmented manner, only once or twice in a paper and are therefore, not considered as relevant.

### 3.3.4. Main remarks

Section 3.3.1

- Summarised outbreak data from zoonoses database (2014–2020) and from literature review (2010–2022) are in line with the EFSA opinion on FoNAO (EFSA BIOHAZ Panel, 2013), although the relative importance of some hazards has increased for *L. monocytogenes, C. parvum* and *Y. enterocolitica*.
- *L. monocytogenes, Salmonella* spp. and human pathogenic *E. coli* can contaminate a wide range of FVHs, have a high impact on morbidity and mortality, and should therefore always be considered in the hazard analysis for ffFVHs.
- Leafy greens (fresh-whole or fresh-cut) were the main vegetable vehicle and associated with many hazards such as pathogenic *E. coli*, noroviruses, *Salmonella* spp., *L. monocytogenes*, *Yersinia enterocolitica* and *C. parvum*.
- Frozen FVHs, especially berries, were common vehicles for viral outbreaks. Additionally, frozen corn was the vehicle for a listeriosis outbreak.
- Other hazard and product combinations causing several outbreaks were sprouts and *Salmonella* spp. and kale and *C. parvum*.
- Outbreak data are often incomplete and reporting varies between countries.
- Investigations can be biased towards types of foods associated with a higher risk and/or known hazards which are more likely to be identified by the healthcare system.
- Large outbreaks and outbreaks of longer duration or associated with serious disease are more likely to be reported and investigated.
- Outbreak data excludes data where the agent and/or product was not identified, often due to the short shelf-life of the FHVs, except for frozen products.
- Outbreak data were the primary resource used to answer this ToR. The uncertainties linked to this database likely provide a relative overestimation of known hazard and product combinations and an underestimation of the relative impact of cases occurring from emerging hazards.

Section 3.3.2:

- Emerging agricultural practices such as hydroponics, vertical farming and urban agriculture, refer to new or increased cultivation practices for raw materials such as fresh fruits, vegetables and herbs which may introduce pathogens into the food chain although the extent of their occurrence is expected to be lower compared to conventional farming activities. Recirculation of water, higher temperature conditions or intense contact between water/commodity have been identified as risk factors.

Section 3.3.3:

- Waterborne hazards linked to the post-harvest use of water in the ffFVH production identified from the literature review of water related papers are:

- Bacteria: *Vibrio* spp., *P. aeruginosa, A. hydrophila, C. perfringens* and *A. butzleri*. These were reported in various papers, linked to various sources of water and can have an oral route of infection.
- Viruses: Adenovirus and Rotavirus could additionally be identified from the water perspective.
- For the protozoa *Giardia* spp. could be identified based on the literature search.
- No conclusion could be made for the helminths (too fragmented and limited literature information available).

- These organisms are considered as established waterborne organisms, however, since these are present in the water, it can be expected that they also can be retrieved on fresh FFVHs. Thus, they might be considered possible future emerging waterborne hazards that require further investigation and surveillance.
- A total of 44 papers were retrieved linked to waterborne hazards. However, most of the hazards were retrieved via targeted analyses. Untargeted analyses would have been more informative when looking for emerging microorganisms. Therefore, attribution of weight to the selected papers is biased.

## 3.4. Criteria to select case-studies for the assessments

A total of 39 processing lines were described within the 31 FBOps that answered the EU industry survey. Responses included commodities from three different sectors: fresh-whole, fresh-cut and frozen FVHs. More than 50% (20/39) of the replies refer to fresh-cut FVHs followed by fresh-whole FVHs (about 38%, 15/39). The less represented sector was the frozen FVHs (10%, 4/39). Among the commodities, leafy greens were the most represented (18 out of 39 processing lines). When analysing the research papers retrieved from the literature search, it was found that most of the research papers selected for the quantitative data extraction with a focus on the use of process water in the post-harvest handling and processing operations of ffFVHs, included leafy greens in their studies, followed by tomatoes and peppers (Figure 7A).

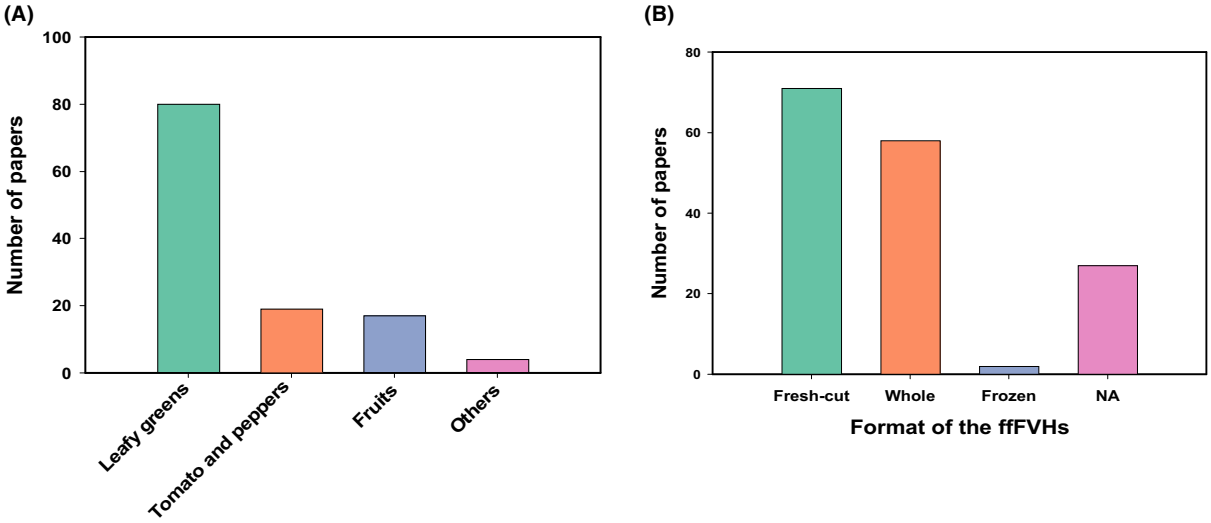



**Figure 7:** Number of research papers extracted from the literature search performed within this scientific opinion indicating (A) a specific type of produce and (B) a specific sector

In the market, there are three main categories of FVHs including fresh-whole, fresh-cut and frozen FVHs. The literature review showed that fresh-cut and whole FVHs have been the focus of the research (Figure 7B). However, very little attention has been given to frozen FVHs. Only two research papers included post-harvest processing in frozen FVHs.. It is only in the last couple of years, after the *L. monocytogenes* outbreak linked to frozen corn, that frozen FVHs have been considered as an important case study to be considered.

The information retrieved from the EU industry survey and the literature review highlights that the case study combining leafy greens and washing operations is the most frequently evaluated by the researchers, mostly due to the high number of outbreaks linked to this type of product. However, it

also highlights current data gaps. The case studies selected for the assessment should include the use of water in post-harvest handling of fresh-whole and frozen FVHs, as there is only limited information available from the literature.

#### 3.4.1. Main remarks

- There are three main sectors where handling and processing operations are relevant for water use. The three sectors are fresh-whole FVHs, fresh-cut FVHs and frozen FVHs.
- Based on the industry survey and the literature review, most of the research papers focus on washing of fresh-cut FVHs, followed by washing of fresh-whole fruits and vegetables.
- Information on frozen FVHs is limited.

### 3.5. Routes of water contamination for microbiological hazards associated with different post-harvest handling and processing operations for ffFVHS (AQ2)

Water contamination routes include both the potential contamination coming from the water source as well as the contamination coming from the FVHs and the environment of the post-harvest handling and processing operations, including FVHs, staff and equipment, among others. Figure 8 illustrates several examples of different post-harvest handling and processing operations for ffFVHs where process water is used in fresh-whole, fresh-cut and frozen FVH sectors.


**Figure 8:** Examples of different post-harvest handling and processing operations for ffFVHs where water is used. Examples include from top to down for whole FVHs: hydroponics grown tomatoes, transport of apples and washing of cucumbers in the processing plant; for fresh-cut FVHs: aquaponics grown leafy greens, de-coring of lettuce and washing of shredded lettuce in the processing plant and for frozen FVHs: hydroponics grown baby spinach at greenhouse, dumping and cooling after freezing of cauliflower in the processing plant

### 3.5.1. Environmental investigations of outbreaks

The literature search retrieved 36 outbreak papers for data extraction, however, only 9 of these papers included environmental investigations in which the contamination source was identified. In six investigations, the contamination most likely occurred at primary production. Examples of contamination during primary production included fresh-cut leafy greens contaminated with *C. parvum* (Åberg et al., 2015) and *E. coli* O145 (Baloch, 2014) respectively. Furthermore, two *Salmonella* outbreaks from fresh-whole fruits have been described, from peppers (Barton Behravesh et al., 2011) and tomatoes (Behravesh et al., 2012). A *Campylobacter* outbreak was caused by fresh peas contaminated by bird faeces in the field (Kwan et al., 2014) and one *E. coli* O157 outbreak where handling of soil contaminated potatoes and/or unwrapped leeks was the most likely vehicle (Launders et al., 2016). Contaminated imported raw produce (from outside EU/EEA) were mentioned as a probable cause in European outbreaks related to norovirus and berries (Mäde et al., 2013; Müller et al., 2015; Einöder-Moreno et al., 2016), *Shigella* spp. and fresh herbs; basil (Guzman-Herrador et al., 2011), curry leaves (Waldram et al., 2018) and mint (Appendix D) and *Salmonella* spp. and curry leaves (Waldram et al., 2018) and melons (ECDC and EFSA, 2021).

The three outbreaks where investigations concluded that the contamination occurred in the processing plant or packing house were all caused by *L. monocytogenes;* on stone fruit (Chen et al., 2016), frozen corn (EFSA and ECDC, 2018a) and leafy greens (Stephan et al., 2015). In the latter, the cause for the product contamination was related to a design-inherent hygienic problem of one specific product-feeding conveyor belt. In an outbreak caused by cantaloupe the possible routes for *L. monocytogenes* introduction were either a truck kept adjacent to the processing line or low-level contamination of incoming cantaloupe, causing the contamination of the equipment (McCollum et al., 2013).

Furthermore, based on the assumption that outbreaks caused by multiple strains are most likely contaminated at primary production, another three outbreaks can be added to the list of primary production contamination; (i) fresh herbs (curry leaves) as the vehicle of *Salmonella* (several serotypes), EAEC, EIEC and *Shigella sonnei* in the United Kingdom (Waldram et al., 2018), (ii) several norovirus genotypes from frozen strawberries indicating wastewater contamination (Mäde et al., 2013) and (iii) serotypes of *Salmonella* (Newport and Reading) from lettuce (Lienemann et al., 2011).

Of the six ROAs published between 2011 and 2021, primary production was the suspected point of contamination for two *Salmonella* spp. outbreaks. The vehicles were melons (from Honduras) and cucumbers (from Spain) respectively. However, no positive samples were retrieved during the back tracing despite extended sampling plans of products as well as the production environment including water (EFSA and ECDC, 2018b; ECDC and EFSA, 2021). Further ROAs included *L. monocytogenes* in corn (EFSA and ECDC, 2018a) and two HAV outbreaks with berries as vehicles. In the latter two no conclusion on the routes of contamination could be established (ECDC and EFSA, 2013, 2014).

### 3.5.2. Occurrence of pathogens at different stages in the production chain

From the literature search, testing of 204,093 FHVs samples were reported at different stages of the production chain. Of the prioritised hazards only occurrence data from more than 300 samples at all three stages (primary production, processing plant and retail) were reported for *Salmonella* spp., *L. monocytogenes* and pathogenic *E. coli*. The general trend for *Salmonella* was a decreasing occurrence from primary production (2.8%) – at the processing plant or packing house (0.99%) to retail (0.48%) (Table 3) which could indicate primary production as the main place of contamination. For *L. monocytogenes* and generic *E. coli*, higher occurrence was reported from the processing plant compared to primary production. Pathogenic *E. coli* (or STEC) was rarely detected (Table 3).

**Table 3:** Occurrence (95% confidence interval) in ffFHV of *Salmonella* spp., *L. monocytogenes* and *E. coli* (STEC and generic) at primary production, processing plants (including packing houses) and retail, e.g. supermarkets, markets and farmers markets summarised from searched scientific literature

| Agent | Primary production | | | Processing plant | | | Retail | | |
|---|---|---|---|---|---|---|---|---|---|
| | Units tested | Positive units | % positive units (95% CI) | Units tested | Positive units | % positive units (95% CI) | Units tested | Positive units | % positive units (95% CI) |
| *Salmonella* spp. | 1,594 | 44 | 2.76% (2.01–3.69) | 2,821 | 28 | 0.99% (0.66–1.43) | 58,524 | 281 | 0.48% (0.43–0.54) |
| *Listeria monocytogenes* | 1,277 | 39 | 3.05% (2.18–4.15) | 14,967 | 1357 | 9.07% (8.61–9.54) | 450,28 | 451 | 1.00% (0.91–1.10) |
| STEC | 801 | 0 | 0% (0–0.46) | 1,972 | 0 | 0% (0–0.19) | 27,578 | 15 | 0.05% (0.03–0.09) |
| *E. coli* (generic) | 800 | 43 | 5.38% (3.92–7.17) | 797 | 56 | 7.03% (5.35–9.03) | NA | NA | NA |

CI: confidence interval.

Table 4 summarises the reported objective sampling from EU monitoring for the above mentioned pathogens as described in Section 2.1.3 but with further merging of sampling stages. No occurrence data were reported in ffFVHs for *Bacillus*, *Cronobacter* and *Echinococcus* according to the selection criteria. Compared to the literature data presented in Table 4, lower occurrence values were reported for *L. monocytogenes* and *Salmonella*, both at primary production and in processing plants, whereas the occurrence values at retail were similar. For the other pathogens listed in the Methods section the number of samples were generally too low to draw any conclusion (Appendix F). For all three pathogens in the presented dataset the occurrence increased along the supply chain from primary production to retail stages. Whether this is a function of mixing and partitioning of batches or bacterial enrichment or contamination at later stages is uncertain.

**Table 4:** Occurrence (95% confidence interval) in ffFVH of *Salmonella* spp., *L. monocytogenes* and STEC from the EU monitoring data (objective sampling) at three different stages of the production chain: (i) primary production, (ii) Processing plants (including sampling at packing centres and wholesalers) and (iii) retail (including all other sampling places described in Section 2.1.3)

| Agent | Primary production[(a)] | | | Processing plant and wholesale[(b)] | | | Retail and catering[(c)] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Units tested | Positive units | % positive units (95% CI) | Units tested | Positive units | % positive units (95% CI) | Units tested | Positive units | % positive units (95% CI) |
| *Salmonella* spp. | 2,971 | 3 | 0.10% (0.02–0.29) | 19,882 | 74 | 0.37% (0.29–0.47) | 71,869 | 356 | 0.50% (0.45–0.55) |
| *Listeria monocytogenes* | 992 | 0 | 0% (0–0.37) | 5,859 | 40 | 0.68% (0.49–0.93) | 20,986 | 220 | 1.05% (0.91–1.20) |
| STEC | 670 | 0 | 0% (0–0.55) | 3,586 | 2 | 0.06% (0.01–0.20) | 12,416 | 19 | 0.15% (0.09–0.24) |

CI: confidence interval.
(a): Results reported for samples collected at 'farm' sampling stage.
(b): Results reported for samples collected at 'processing plant', packing centre' and 'wholesale' sampling stages.
(c): Results collected for samples collected at 'retail', 'catering', 'restaurant or café or pub or bar or hotel or catering service', 'school or kindergarten' and 'hospital or medical care facility' sampling stages.

There are significant differences between data obtained from the literature review and data obtained from the EU monitoring for the primary production and processing plant. The reason for these differences is not known. Different hypotheses exist, such as the type of sampling or detection methodology. On the other hand, occurrence data obtained at retail was similar for the three hazards

when comparing the two sources of information. There were no data on pathogen levels. The higher occurrence at retail may be a consequence of processes such as mixing, cross-contamination and partitioning, e.g. from one heavily contaminated lettuce head (or batch), present in a batch characterised by a low bacterial occurrence, bacteria can cross-contaminate other lettuce heads (or following batches) during mixing, leading to higher occurrence, but lower levels of bacteria per lettuce head/batch, when partitioned (Danyluk and Schaffner, 2011).

### 3.5.3. Main remarks

- Hazards are expected to contaminate FVHs during primary production, especially the zoonotic hazards transmitted via the faecal-oral route, e.g. *Salmonella* spp., STEC, *Yersinia* spp. and *C. parvum*.
- For *L. monocytogenes* most studies suggest contamination takes place mainly at the processing plant or packing house. It should be noted that it is possible that the raw products are harbouring the bacteria entering the processing plant in low, undetectable, numbers and that some specific strains thereafter are accumulated in the process water or can form biofilms and contaminate surfaces within the processing plant, e.g. as indicated in the frozen corn outbreak.
- There was less information on viral numbers and contamination routes, however food-borne viruses can contaminate the product all along the production chain, most often from manual handling.
- Hazard occurrence data along the production chain did not provide evidence for a specific point of contamination for any hazard.
- Imported raw produce is more likely to be associated with specific hazards, e.g. *Shigella* spp. and enteric viruses (HAV).
- Depending on the operation, all hazards can potentially be accumulated in the process water and lead to batch-to-batch cross-contamination.
- Few environmental outbreak investigations have identified the contamination route.
- No longitudinal studies were found which were designed to specifically investigate the contamination route, i.e. taking a representative number of samples of the same batches of FVHs at different stages from farm to fork.
- Hazard occurrence data was aggregated from different FVH food categories at three stages of the food chain (primary production, processing plant, retail). This aggregation may have influenced the observed results for the combination of a microbiological hazard and a specific food category.
- The data is unbalanced, with more samples taken from retail compared to primary production and processing plants/packing houses.

## 3.6. Contamination rates for the most relevant microbiological hazards in process water in different post-harvest handling and processing operations of ffFVHs and between different ffFVHs batches (AQ3)

In this opinion, the **contamination rate of process water** (from product to water) is defined as the change (usually the increase) of the microbial load (of microbiological hazards identified in TOR 1.1) in process water per unit of time. In the absence of any water disinfection treatment and replenishment strategy, the numbers of microbial cells in the process water at any given time represent the microbial load associated with the water source as well as microorganisms from soil, debris and dust coming from the product to the water as well as the cells detached from ffFVHs surface transferred to the water. The implementation of water disinfection treatments and water replenishment strategies (periodically or continuously) have an impact on the contamination rate of the process water depending on the microbial survival against the added biocide and the dilution caused by replenishment.

It should be considered that the increase in the microbial load of the process water may also result from bacterial cell growth on organic matter (e.g. soil, plant exudates, etc.) or suspended in water, depending on the duration of the process, the temperature and the water replenishment rate. For instance, when process water is used for several weeks at ambient temperature (e.g. apples) or if it is replenished at low frequency, thus remaining for a long time in the washing tank accumulating a high level of organic matter. In most of the cases, process water used in the different operations

(e.g. washing tank, fluming, hydrocooling) is fully replenished at least once a day or more. In these cases, the contribution of microbial growth in the increase of cell numbers in water is considered negligible compared to the increase due to the detachment of cells from incoming product. Similarly, the quantitative contribution of microorganisms transferred from the equipment surface to water can be assumed to be not important in relation to the number of microorganisms detached from the product or introduced with the water source, the soil, debris, dust, etc. Based on the definition above, the contamination rate of process water depends on multiple variables, including the proportion of produce that is contaminated, the load of microorganisms in the contaminated produce, the produce: water ratio (w:v) (Smolinski et al., 2018; Possas and Pérez-Rodríguez, 2023) as well as the intervention strategies put in place (e.g. water replenishment and disinfection treatments) and the transfer of microorganisms from product to water. In practice, contamination levels rapidly reach a 'steady state', i.e. the number of cells exiting the tank *via* partial removal (with the water and/or product going out the tank), the dilution effect *via* partial replenishment of water, and/or inactivated by biocidal products, is of similar magnitude to the number of cells detached from incoming product. Nevertheless, the equilibrium can be easily perturbed if (i) microbial accumulation exceeds the rate of incoming cells via new batches, (ii) the conditions related to time, temperature and organic matter favour microbial growth (highly improbable, but possible under flawed hygiene conditions) or (ii) there is no intervention strategy in place to avoid accumulation of microorganisms in process water (e.g. in case water replenishment or available biocides become insufficient to counteract the microbial accumulation), and thus, microbial contamination in the water tank may increase over time.

Similarly, the **contamination rate of the product being processed** (from water to product) is defined as the change of the microbial load in the product per unit of time, mainly because of the attachment of microorganisms from process water to the product while being processed. The contamination rate of the product depends on the contamination present in the process water, the ratio produce (weight):water (volume) (w:v) as well as the intervention strategies applied and the transfer of microorganisms from water to product.

In both cases, the value of the contamination rate may occasionally be negative (i.e. '*decontamination rate*'); for instance, for time spans when more cells are inactivated by the disinfection treatment than are transferred to water or to produce.

The transfer of microorganisms are determined by many factors related to the microbial transfer from product to water and *vice versa* from water to product such as:

- type of commodity including vegetable species, botanical variety and plant surface characteristics such as hydrophobicity and roughness. It has been described that specific surface properties such as hydrophobicity, electrical charge and surface roughness affect the transfer ability from product to water and *vice versa*. A high hydrophobicity and surface roughness contribute to limited bacterial-removal during washing (Ukuku and Sapers, 2001; Possas and Pérez-Rodríguez, 2023).
- condition of commodity, e.g. presence of soil, leaves, initial microbial contamination.
- implicit factors of the microorganism (e.g. colonisation and/or internalisation potential, the impact of the physiological state on internalisation, resistance to inimical factors on product surface and disinfectants, ability to attach/detach, etc.).
- type of handling and processing operations of the ffFVHs (e.g. washing, cooling, etc.) and how these are implemented, including the intensity of the physical forces (e.g. compressed air applied during washing) affecting the detachment of the microorganisms and potential attachment.
- factors related to the water characteristics including temperature of the water during processing, organic load in the water, water disinfection treatment and contact time, etc.

The literature search outcome allowed the calculation of transfer of microorganisms from lab-scale experimental data. The resulting calculations showed a wide range of transfer values between studies (from less than 1% to ~ 100%) and in some cases also within the same study (Allende et al., 2008b; López-Gálvez et al., 2009; Holvoet et al., 2012). The variability could be related to differences in the experimental set up, microorganisms, amount and type of organic matter, as well as the error of the analytical enumeration method, since the microbial populations used for the calculations of transfer coefficients were in some cases close to the enumeration limit.

It should be noted that the calculations of transfer of microorganisms are only examples that reflect a process-specific parameter and thus, may vary with different processing scenarios and hazard-produce combinations. Moreover, when expressing the transfer of microorganisms as %, the actual

number of cells transferred depends on the total number of bacteria present. As such, transfer can have a limited impact on the accumulation of microbial contamination in water, regardless of the magnitude of the transfer coefficient. For instance, a high-percentage value may be associated with a low total number of transferred bacteria whereas, a low value may be associated with a high total number of transferred bacteria.

The contamination rate during ffFVH post-harvest operations has been addressed through modelling approaches. Munther and Wu (2013) and Munther et al. (2015) proposed two systems of equations that describe the changes over time in the population density of *E. coli* O157:H7 in process water as well as on the produce based on the transfer rates from product to water and from water to product. These approaches also take into account the impact of different intervention strategies on water chemistry (e.g. the levels and frequency of chlorine addition causing pathogen inactivation, the average time of the produce in the tank, changes in COD and its impact on chlorine de-activation, etc.).

Despite the available information outlined above, existing models cannot readily simulate the contamination dynamics of all possible post-harvest handling and processing operations, mainly because they are 'informed' by (=fitted to) lab-scale, batch or static data, regarding product contamination and specific intervention strategies. As such, substantial amendments and customisations in the model structures and assumptions are needed for tailoring the models to different industrial processes.

The final objective of the work performed within this working group is the elaboration of sector specific guidelines and the development of a user-friendly tool for FBOp to allow them to manage different intervention strategies (e.g. water replenishment and/or biocides charging of the system) aimed to avoid or minimise cross-contamination of produce by process water. Therefore, it is critical to understand which factors determine the contamination rate of the process water in a processing operation and how this can be approximated by experimental data. For instance, a preliminary assessment of water contamination dynamics and water/biocide replenishment rate, would increase the understanding of the specificity of each process and assist in the mathematical simulations. Model outputs may assist in setting operational conditions (e.g. process criteria) to maintain the lowest possible microbial contamination in water (performance objective) and validate their performance. This will be elaborated further in coming opinions of this mandate.

### 3.6.1. Main remarks

- The contamination rate of process water is defined as the change of the microbial load in process water per unit of time, and is determined by multiple variables such as the type of product entering the operation where water is used, the microbial cells that detach from the product (i.e. that are released in the water) and the survival of microorganisms in the process water.
- The contamination rate of the product being processed is defined as the change of the microbial load on the product per unit of time.
- The contamination rate of process water and the processed product depends on multiple variables, including the type of FVH and its condition, the ratio produce:water (w:v), the intervention strategies (disinfection and replenishment) put in place, as well as the transfer of microorganisms from product to water and vice versa from the water to the product surface.
- The transfer of microorganisms between water and product and vice versa, are influenced by many factors such as type of handling and processing operations, type of commodity, implicit factors of the microorganism and factors related to the water characteristics.
- Available quantitative data to calculate the transfer of microorganisms between product to water and water to product are scarce and show high variability. It is not possible to define a single value for the transfer of microorganisms that applies for all types of hazard-produce combinations and processing conditions.
- The transfer of microorganisms from product to water and vice versa can also be estimated by fitting mathematical models to a given experimental data set, which may also reflect different conditions during washing (e.g. type of microorganisms, commodity, such as wash time, produce wash rate, volume of the wash tank, etc.).
- Data obtained under industry conditions will be used in combination with optimised mathematical models to describe different scenarios in three different sectors (whole, fresh-cut and frozen FVHs) and to provide guidance on the impact of implementing different intervention strategies. These results will be elaborated further in coming opinions of this mandate.

## 3.7. Water management plan and system

Based on the available literature from studies performed at industrial scale (see Section 3.6), it becomes clear that if the process water is not well managed, the accumulation of indicator organisms and/or pathogens in process water is occurring and potential cross-contamination to produce is present.

Fit-for-purpose water quality can be defined (i.e. level of indicator organisms in the process water) as part of the FSMS (food safety management system) of a FBOp. Therefore, a shift from water controlled by a PRP to a HACCP-based approach to govern quality of process water, recalled as 'water management plan' (WMP) and 'water management system' (WMS) is necessary (Figure 9). An example of the steps to be taken in the water management plan can be found in (WHO, 2017). A plan becomes a system when operationalised in practice, including validation, operational monitoring and verification. A WMP consists of two pillars, the preventive measures based on GHP/GMP (Section 3.8) and water management strategies, as interventions to reduce the microbiological load of the process water (Sections 3.9, and 3.10). When interventions are set, and water management strategies are introduced, a validation and verification is needed to demonstrate their performances (Section 3.11) and an operational monitoring, as part of the daily follow-up of a production (Section 3.12) (FAO and WHO, 2021b).

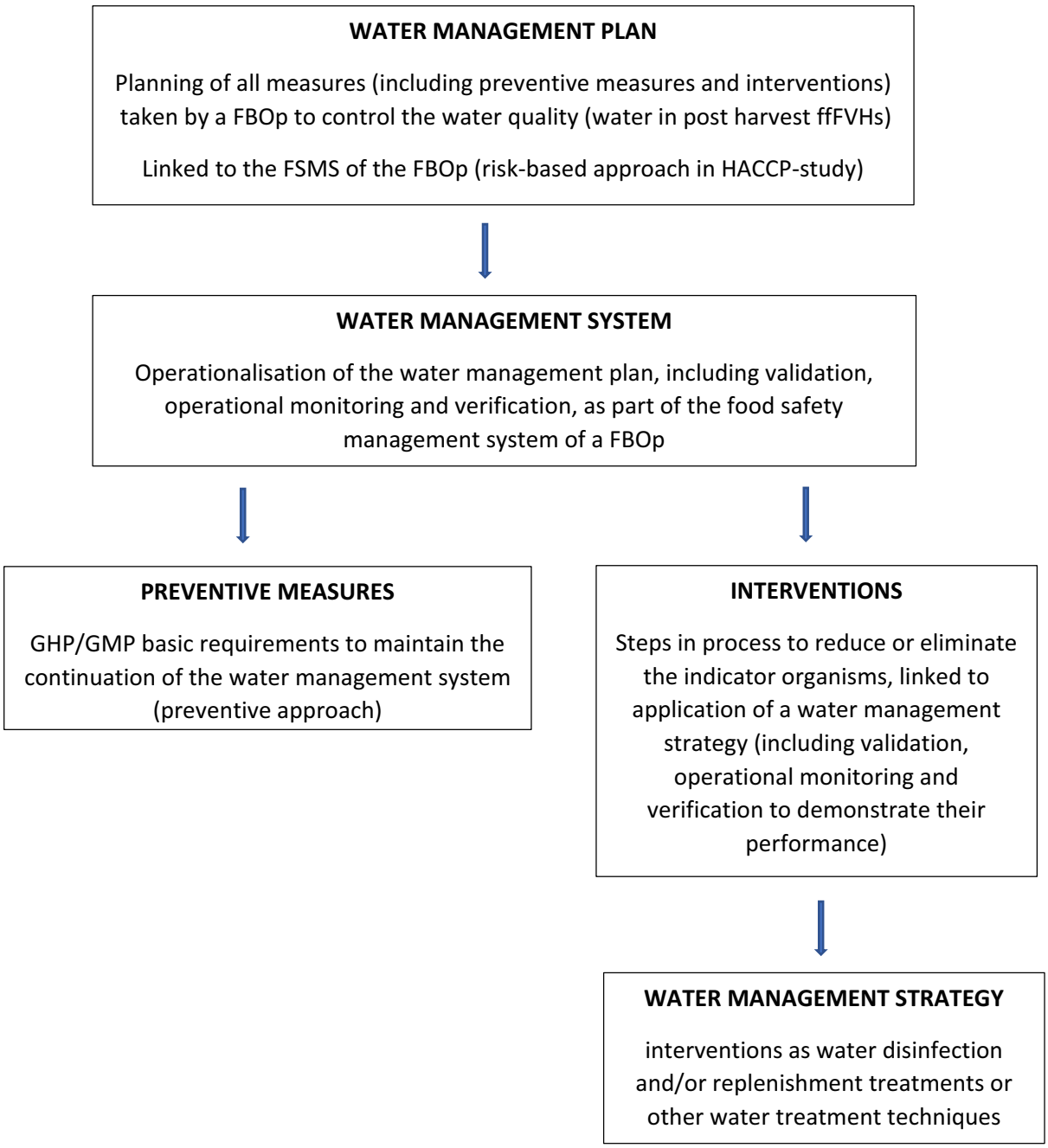



**Figure 9:** Water management plan and implemented water management system based upon preventive measures and interventions to illustrate the risk-based approach in fit-for-purpose water applied in post-harvest activities of ffFVHs

### 3.7.1. Main remarks

- Fit-for-purpose water quality can be achieved (i.e. level of indicator organisms in the process water) as part of the FSMS of a FBOp. Therefore, water control based solely on a basic prerequisite programme (PRP) is no longer feasible and a HACCP-based approach is also required as part of the water'water management plan' and 'water management system'.
- The water management system is based on two complementary pillars: (1) preventive measures (good hygienic and manufacturing practices) and, (2) interventions (water management strategies) which must be validated, monitored and verified during use.

## 3.8. Preventive measures: good hygiene and good manufacturing practices in water management, distribution and storage systems (AQ4)

Good practices are preventive measures taken by the FBOp in the frame of their GHP/GMP and their FSMS in general, to prevent contamination of the foods being processed. In this section, GHPs/GMPs linked to the management of water in post-harvest activities of ffFVHs are addressed with the focus on microbiological contamination. Generic GHPs/GMPs not specifically targeting water or water management are excluded. Firstly, relevant legal and international guidance documents were identified (Table 6). Secondly, the most relevant (water related) good practices included in the reference documents with respect to their significance as preventive measures in relation to water management in ffFVH production, were selected based on the expert knowledge.

Good practices, identified as preventive measures in the frame of water applied in post-harvest handling and processing of ffFVHs, to avoid microbiological proliferation are:

- **Technical maintenance of infrastructure associated to water management systems** including:
  - Technical maintenance of water treatment equipment, distribution and storage systems (pipes, tanks, etc.).
  - Calibration of monitoring systems for water management strategies.
  - Cleaning and disinfection of water treatment equipment, distribution and storage systems (pipes, tanks, etc.).
  - Replacement of water distribution systems to avoid contamination due to biofilms.
  - Search for and evaluation of biofilm formation in water tubing systems/water distribution systems.
- **Training staff** in operational monitoring of water management systems
- **Cooling of post-harvest process water** to reduce bacterial growth in the water

Each of the 13 documents, listed in Table 5 and renamed A till M, were screened to have an overview of the relevant good practices included in these documents. A qualitative appraisal was performed based on the level of detail given in each of the guidelines for each identified good practice. Table 6 shows the qualitative appraisal of the identified preventive measures, assigned as 'absent', 'generic description' or 'detailed description'.

**Table 5:** List of legal documents and (international) guidance documents included in the identification of relevant good hygienic practices related to the use of water or management of water in ffFVHs post-harvest production

| Reference code for use in this section | Reference |
|---|---|
| A | FAO and WHO (2021a) |
| B | FAO and WHO (2019) |
| C | FAO and WHO (2008) |
| D | Codex Alimentarius, General principles of food hygiene, CXC 1-1969[(a)] |
| E | Codex Alimentarius, Code of hygienic practice for fresh fruits and vegetables, CXC 53-2003 |
| F | Codex Alimentarius (2022)[(b)] |
| G | Regulation (EC) No 852/2004[(e)] |
| H | Directive (EU) 2020/2184[(f)] |
| I | Commission notice (2017/C 163/01)[(g)] |
| J | Commission Notice (2016/C 278/01)[(c)]<br>Commission Notice (2022/C 355/01)[(d)] |
| K | U.S. FDA (2018) |
| L | U.S. FDA (2017) |
| M | ICMSF (2011a,c, 2018) |

(a): CAC (Codex Alimentarius Commission), 1969. General principles of food hygiene. CXC 1-1969. Adopted 1969. Revision 2020. p. 1–35.
(b): Guidelines for the safe use and re-use of water in food production and processing (General Section and Annex I on Fresh Produce) Report of the 53rd session of the Codex Committee on Food Hygiene, San Diego, United States of America, 29 November-2 December 2022 and 8 December 2022 (report adoption) (at step 5/8), https://www.fao.org/fao-who-codexalimentarius/sh-proxy/zh/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-53%252FReport%252FREP23_FHe.pdf
(c): Commission Notice (EC) No 2016/C 278/01 of 30 July 2016 on the implementation of food safety management systems covering prerequisite programmes (PRPs) and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses. OJ C 278, 30.7.2016, p. 1–56.
(d): Commission notice on the implementation of food safety management systems covering Good Hygiene Practices and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses (2022/C 355/01). 16.9.2022, p. 1–58.
(e): Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1–23.
(f): Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast) (Text with EEA relevance) OJ L 435, 23.12.2020, p. 1–62.
(g): European Commission, 2017. Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (2017/C 163/01). OJ C 163, 23.5.2017, p. 1–40.

**Table 6:** Qualitative appraisal of the level of detail of relevant good practices to prevent microbiological contaminations in water management system in the ffFVHs production given in the selected legal and guidance documents (capital letters refer to the relevant documents in Table 5)

| Good practice | Absent | Generic description | Detailed description |
|---|---|---|---|
| **Technical maintenance of infrastructure associated to water management systems** | | | |
| Technical maintenance of water treatment equipment, distribution and storage systems | B, C, M | D, E, F, G, J, K, L | A, H, I |
| Calibration of monitoring systems for water management strategies | B, C, F, G, H, K, M | A, D, E, I, J | L |
| Cleaning and disinfection of water treatment equipment, distribution and storage systems | C, F, H, I, K, M | A, D, E, G, L, J | B |
| Replacement of tubing to avoid contamination due to biofilms | A, B, C, D, E, F, G, I, J, M | H, K | L |
| Search for and evaluation of biofilm formation in water tubing systems/water distribution systems | A, B, D, E, F, G, H, J, M | I, K | C, L |
| **Training** | | | |
| Training staff in operational monitoring of water management strategies | A, H, K, M | D, F, G, I, J, L | B, C, E |
| **Cooling** | | | |
| Cooling of post-harvest process water to reduce bacterial growth in the water | B, D, F, G, H, I, J, L, M | K | A, C, E |

All evaluated items in legal and guideline documents are further discussed in detail below. This overview can be seen as the set of best practices related to GHP and GMP as preventive measures that can be taken by the FBOp in the frame of their water management plan, to maintain their water management system and to prevent contamination of the foods being processed. Based on the replies provided by the industry survey, it was also assessed if these good practices were applied in practice or not. A brief descriptive analysis of the retrieved answers from a total of 31 industries, is included in each good practice section.

### 3.8.1. Technical maintenance of infrastructure associated with water management systems

In the EU Commission Notice (2017/C 163/01)[3] it is recommended that installation, routine inspection and maintenance of equipment such as backflow devices and air gaps are needed, to prevent contamination of clean water with potentially contaminated water (such as between potable water fill lines and dump tank drain lines). The FAO and WHO report (FAO and WHO, 2021a) indicates that

insufficient cleaning and disinfection of washing baths, combined with a low water replenishment rate due to the use of high product/water ratios lead to a rapid increase in *E. coli* in the process water, with subsequent potential for *E. coli* transfer to the end product (Gombas et al., 2017). In the new drinking water legislation (EU Directive 2020/2184)[9], detailed information is provided on Annex II (monitoring) part A section 2: Monitoring programmes established pursuant to Article 13(2) shall include one or a combination of the following: (a) collection and analysis of discrete water samples; (b) measurements recorded by a continuous monitoring process. In addition, monitoring programmes may consist of: (a) inspections of records of the functionality and maintenance status of equipment; (b) inspections of the abstraction area and of the treatment, storage and distribution infrastructure, without prejudice to monitoring requirements. The Codex Committee on food hygiene (at step 3) states in Appendix IV, section 1 'water fit-for-purpose assessment' on a generic level that 'Additional factors to be considered could include water storage and distribution, including the hygienic design and the need for special expertise', however no more details are further discussed on how to achieve this. In the replies provided to the industry survey, 30 out of 31 industries (97% of industries) indicated that they apply technical maintenance of the infrastructure associated with water management systems.

#### 3.8.1.1. Calibration of equipment of monitoring systems for water management strategies

In the U.S.FDA document 'Draft Guidance for Industry: Standards for the Growing, Harvesting, Packing and Holding of Produce for Human Consumption' a statement is made towards calibration of applied apparatus: 'The extent to which an instrument or control will maintain its accuracy and precision between calibrations depends on the type of instrument; instrument quality; frequency of use; use and storage environment (e.g. high humidity and temperature can affect some instruments and controls); and the way the instrument is used (e.g. rough handling can affect instruments and controls). In some instances, you should periodically compare results against a more reliable instrument (e.g. periodic checks with a pH probe when typically using pH test strips), especially when there is potential for human error or judgement associated with qualitative results (e.g. colour change in a pH test strip) that your instruments and controls are functioning properly. In some cases, you can check the accuracy and precision of your instruments and controls yourself. Generally, accuracy checks involve comparing an instrument's displayed measurement against at least one true value.'

Some other documents also touch upon calibration, however the information provided is not so detailed:

In the general principles of food hygiene CXC 1-1969 (2021)[12], calibration is mentioned as part of verification programmes: Verification, which includes observations, auditing (internal and external), calibration, sampling and testing, and records review, can be used to determine if the HACCP system is working correctly and as planned, but not specific on water management strategies.

In the Commission Notice on the implementation of FSMS covering PRPs and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses (2022/C 355/01)[10] a PRP is formulated regarding 'Technical maintenance and calibration', however, not specifically addressing equipment and monitoring devices applied for water management strategies: 'Technical maintenance and calibration c) Calibration of monitoring devices (e.g. weighing scales, thermometers, flow meters) is of importance in controlling food safety and hygiene'.

In the replies provided to the industry survey, 21 of 31 industries (68% of industries) indicated to perform calibration of monitoring systems for water management strategies.

#### 3.8.1.2. Cleaning and disinfection of water treatment equipment, distribution and storage systems

In the FAO and WHO report (FAO and WHO, 2019), it is stated that water storage tanks and their hygienic maintenance should be included in relevant sanitation schedules. The rest of the revised documents include cleaning and sanitation of the broader infrastructure and equipment of a ffFHVs facility, but not particularly on the water management systems applied in the post-harvest activities of ffFVHs.

In the EU Commission Notice (2017/C 163/01)[3] it is indicated that water delivery systems including basins, tanks and storage of water sources should be maintained and cleaned appropriately, to prevent

[12] CAC (Codex Alimentarius Commission), 1969. General principles of food hygiene. CXC 1-1969. Adopted 1969. Revision 2020. p. 1–35.

microbial contamination of water and biofilm formation. It is also recommended as important risk reduction measures to protect the water source and distribution systems from contamination e.g. from animal and human activity and from surface water entry (FAO and WHO, 2021a). Further information on risk reduction measures such as managing microbial water quality in piped distribution systems, can be found in the literature (WHO, 2017).

In the replies provided to the industry survey, 29 of 31 industries indicated that they are performing cleaning and disinfection of their water treatment equipment, distribution and storage systems.

#### 3.8.1.3. Replacement of water distribution systems to avoid contamination due to biofilms

In the 13 reviewed documents, only one specific requirement is formulated regarding the seeking and destruction of biofilm: in the U.S.FDA document (Draft Guidance for Industry: Standards for the Growing, Harvesting, Packing and Holding of Produce for Human Consumption), water distribution systems are particularly addressed as source of contamination and preventive maintenance on those are required.

The U.S.FDA guidance document stated generically in the section on 'Plumbing system for water in a sprout operation': 'The plumbing system within your sprout operation must be of an adequate size and design and be adequately installed and maintained to distribute water under pressure as needed, in sufficient quantities, in all areas were used for covered activities, for sanitary operations or for hand-washing and toilet facilities (§ 112.133(a))'.

In the replies provided to the industry survey, only 6 of 31 industries (19% of the industries) indicated that they are proactively replacing (parts of) their water distribution systems to avoid biofilm formation.

#### 3.8.1.4. Search for and evaluation of biofilm formation in water tubing and distribution systems

Information regarding the seeking for biofilms were found in two documents: (1) the FAO and WHO report (C) (FAO and WHO, 2008) where reference is made towards the search of biofilm in water tubing systems and (2) the U.S.FDA document (K) 'Draft Guidance for Industry: Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption', where it is stated that 'Periodically performing cleaning and disinfection procedures, including deep cleaning and disinfection procedures, can increase effective soil removal, biofilm prevention and removal, and pathogen inactivation', from the rest of the 13 documents, no specific information was retrieved.

In the replies provided to the industry survey, 13 of 31 industries (42% of the industries) indicated that they are proactively seeking for biofilm formation.

### 3.8.2. Training staff in operational monitoring of water management strategies

On training of staff on the proper functioning of water management strategies, including the operational monitoring, the FAO and WHO report (FAO and WHO, 2019) (B) declares that: 'Where contact is controlled through logistics and staff training, active management and monitoring of required performance at timely intervals will be required to provide the food operation with the confidence that there is no breach of product safety in daily operations'. Also, in the FAO and WHO report (FAO and WHO, 2008) (C) a similar formulation is found (Chapter 9: Education and training). On the other hand, in the Codex Committee on food hygiene (F), attention is drawn to needs for specific expertise.

In the replies provided to the industry survey, 22 of 31 industries (71% of the industries) indicated that staff is trained in the monitoring of water management strategies.

### 3.8.3. Cooling of post-harvest process water to reduce bacterial growth in the water

In the FAO and WHO report (FAO and WHO, 2008) and code of hygienic practice for fresh fruits and vegetables (CAC/RCP 53 - 2003)[4] document, the importance of cooling of post-harvest process water is mentioned in many different sections of the document. In general, water-based cooling methods have the potential to transfer pathogen contamination from product to water/ice and water/ice to product, unless water/ice quality is effectively controlled by disinfection and regular monitoring. Cooling water can be recirculated provided water quality is similarly maintained. Microbial quality of ice should be also considered to avoid potential contamination (FAO and WHO, 2021a).

**In the replies provided to the industry survey**, only 8 of 31 industries (26% of the industries) indicated that they are cooling the water applied during processing.

### 3.8.4. Main remarks

- Good practices (GHPs/GMPs) as preventive measures as part of water management plans/ systems applied in post-harvest handling of ffFVHs identified are:
    - Technical maintenance of water management strategies (water disinfection and/or replenishment/refreshment) including:
        - Preventive technical maintenance of equipment and installations.
        - Calibration of monitoring systems for water management strategies.
        - Cleaning and disinfection of water treatment systems and water distribution (pipes, tanks, etc.).
        - Replacement of water distribution systems to avoid contamination due to biofilms.
        - Search for and evaluation of biofilm formation in water tubing systems/water distribution systems.
    - Training staff in operational monitoring of water management systems.
    - Cooling of post-harvest process water to reduce bacterial growth in the water.
- A total of 13 guidelines, and legislation from global, international and European perspectives have been qualitatively screened for the presence of these good practices and the level of technical detail, so that a FBOp can apply this information in their water management plan/ system. However, none of the documents was suitable for all identified good practices.
- A set of best practices was formulated for a FBOp to implement as preventive measures to guarantee the good functioning of the applied water management strategies.
- More specific and detailed information is maybe available from national guidelines and/or regulation; however, these have not been included in the literature study.
- From the replies provided to the industry survey, it became clear that three good practices are not yet well implemented in practice: (i) replacement of infrastructure to avoid biofilm formation, (ii) seeking for biofilm formation in the water management system and (iii) cooling of the water.
- The good practices applied by most of the responding EU food industries were: (i) technical maintenance, (ii) cleaning and disinfection, (iii) training of staff and (iv) calibration of monitoring equipment.

## 3.9. Water disinfection systems (AQ5)

The term water disinfection treatments describe the different treatments including biocides and physical disinfection treatments used to maintain the microbiological quality of the process water with the purpose of avoiding cross-contamination of ffFVHs. Within the literature, biocides used to maintain the microbiological quality of process water are defined as disinfectants or sanitisers. Although slight differences exist in the degree of efficacy between disinfectants and sanitisers, both terms are used interchangeably in the current literature. The definitions for water disinfection treatment, biocide, sanitiser, disinfectant and efficacy are included in the glossary of this scientific opinion. Based on the Codex Alimentarius, a biocide is a chemical substance or microorganism intended to destroy, deter, render harmless or exert a controlling effect on any harmful organism by chemical or biological means (CAC/RCP 53 - 2003).[4] In the EU legislation, biocidal products mean 'any substance or mixture, in the form in which it is supplied to the user, consisting of, containing or generating one or more active substances, with the intention of destroying, deterring, rendering harmless, preventing the action of or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action' (EU, 528/2012)[13]. The EU Biocidal Products Regulation (EU, 528/2012)[21] classifies the different biocidal product-types in four groups and the uses foreseen within each group are described. Disinfectants constitute the Group 1, and comprise the following uses: (i) human hygiene, (ii) disinfectants and algaecides not intended for direct application to humans or animals, (iii) Veterinary hygiene, (iv) food and feed area and (v) drinking water. Based on the EU directive on the

[13] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products Text with EEA relevance. OJ L 167, 27.6.2012, p. 1–123.

quality of water intended for human consumption (EU, 2020/2184)[9], water intended for human consumption includes all water used in any food business for the manufacture, processing, preservation or marketing of products or substances intended for human consumption. Therefore, biocidal product-types authorised to be used for the disinfection of drinking water, can be used to maintain the microbiological quality of water use in the post-harvest handling and processing operations of ffFVHs. However, it should be considered that in accordance with the EU Biocidal Products Regulation (EU, 528/2012)[13], Member States are allowed to restrict or ban the use of biocidal products in the supply of drinking water to the public, including in individual supplies. In some MSs (e.g. Spain) the use of several biocides has been assessed within the national authorisation framework for use as processing aids (TRIS-European Commission, 2023).[14] It should be considered that the use of biocidal products when used as processing aids are not regulated as such at EU level because processing aids are outside the scope of the EU Biocidal Products Regulation (EU, 528/2012).[13] Therefore, any decision linked to the use of biocides as processing aids to maintain the microbiological quality of process water is expected to be made by each MS. In addition, it is also expected that the conditions required to apply a water disinfection treatment as biocide or as processing aid might be different.

Among the water disinfection treatments that can potentially be used to maintain the microbiological quality of process water, are chemical, physical, bacteriologically based and/or combinations thereof. The advantages and disadvantages of these various disinfectants have been summarised in the scientific literature (Gil et al., 2009; Ölmez and Kretzschmar, 2009; Gombas et al., 2017; Raffo and Paoletti, 2022). However, the application and reports concerning the use of water disinfectants in the post-harvest setting beyond that of a lab-scale are limited (Ali et al., 2018), suggesting that the results on the industrial or commercial-specific conditions of such applications in the food industry are rather limited (Bilek and Turantaş, 2013; De Corato, 2020).

### 3.9.1. Most common disinfection treatments used to maintain the microbiological quality of process water (AQ5/SQ5)

Among the responses obtained from the 31 industry survey replies, 92 processing lines were described for various ffFVHs. The majority of the survey respondents (76%) reported using biocides, while the remaining (24%) reported no biocide use. Of the processing lines using biocides, 55% (i.e. 51 of the 70 lines) used some form of chlorine (gas, aqueous, combinations of chlorine-based biocides and physical methods, etc.), followed by the use of some form of PAA (16%), $H_2O_2$ (12%) and ozone (1%). Notably, the use of PAA or $H_2O_2$ was reported in 10% (i.e. 7 of the 70 lines) of processing lines.

Overall, the literature search supported the results obtained from the industry survey, indicating that chlorine-based biocides are the most commonly used biocides by the European industry, followed by PAA (Warriner and Namvar, 2013; Banach et al., 2015, 2021; Meireles et al., 2016; Gil, 2021; Raffo and Paoletti, 2022). A few reasons for the limited application of other water disinfection techniques, although some show the capacity and potential to inactivate food-borne pathogens, are technical limitations when applied on an industrial scale, high operation costs compared to current disinfectants, consumer concerns and limitations on the ability to eliminate internalised microorganisms (Allende and Gil, 2014).

When considering alternative disinfection methods to chlorine and PAA, Meireles et al. (2016) noted that those specific to post-harvest water include ozone, ultrasound, the combination of UV and ozone, and the use of quaternary ammonium compounds (QACs). Other antimicrobial treatments, like bacteriophages and natural compounds like essential oils, are receiving more attention. Still, the application beyond that of the lab scale and demonstrated efficacy as a water disinfectant treatment in an industrial setting has not yet been shown (López-Gálvez et al., 2021). Other alternatives reported in the literature include cold or non-thermal plasma, electrolyzed water and a combination of chemical and/or physical methods.

### 3.9.2. Physico-chemical parameters of process water with an impact on the efficacy of the most used disinfection treatments (AQ5/SQ6)

Process water is commonly recirculated and recycled for economic and environmental considerations. During the washing process, only a limited amount of fresh water is added to the wash

[14] TRIS (Technical Regulations Information System – European Commission), 2023. Technological adjuvants in food, Notification number 2023/0114/E (Spain), https://technical-regulation-information-system.ec.europa.eu/en/notification/23295

system to compensate for water loss during washing. Water re-circulation and high throughput of ffFVHs also allows steady organic matter accumulation and accelerated biocide depletion. Maintaining effective biocide concentrations in process water is critical for the efficacy of the disinfection treatment (Simons and Sanguansri, 1997; Gil et al., 2009; Luo et al., 2012). Most of the scientific papers retrieved showed that the disinfection efficacy is closely associated with physico-chemical parameters of process water. Among the retrieved literature (159 papers), 80 papers studied factors impacting chlorine efficacy with 26 papers for PAA.

Free chlorine (FC) level in the prewash water is rapidly depleted by organic/inorganic material during produce washing, and therefore needs regular dosing. The industrial scale application and management of chlorination is arduous, needing monitoring and control of the FC level in the process wash water to minimise microbiological and chemical risks (Raffo and Paoletti, 2022). Industry guidelines for process water management recommend maintaining a sufficient level of residual FC instead of a target prewash total chlorine level (Gombas et al., 2017; IFCPA and others, 2006; Arizona LGMA, 2017; California LGMA, 2017 cited in Fu et al. (2018)). Measuring FC alone may be a poor indicator of the efficacy of the disinfection if other physico-chemical parameters (for instance, turbidity, COD and pH) are ignored. However, organic load rarely impacted ($p > 0.05$) the efficacy of either PAA or mixed peracid, with typical reductions of > 5 log CFU/mL in process water throughout processing for all organic loads (2.5, 5 and 10%) (Davidson et al., 2014). To maintain a steady biocide level, stock biocide solution is frequently added to the wash system during the replenishment process, manually or automatically, based on feed-back control. A tomato dump tank water study (Zhou et al., 2014) showed that, during manual replenishment control, the FC concentration varied from undetected to 150 mg/L, but under automatic control, the level of biocide can be kept within the consistent level of 20 mg/L. Another study showed (Luo et al., 2018) that FC level in leafy greens process water fluctuated within 10 mg/L under automatic control (continual monitoring and stock solution injection) but depending on product types. This study also showed that the FC level of 10 mg/L is a critical point under which most of natural/background microbiota (including spore-forming microorganisms) survives and therefore accumulates with the new produce washed in the same process water. Another study showed that, during chlorine depletion and chlorine replenishment processes, no pathogen survival was observed when the level of FC in the process water was maintained above 3.66 mg/L, irrespective of the initial FC levels (10, 50, 100 and 200 mg/L) or organic loading (chemical oxygen demand (COD) levels of 0, 532, 1,013 and 1,705 mg/L). At this FC concentration, the measured oxidation–reduction potential (ORP) was 843 mV and pH was 5.12 for the chlorine depletion process; the measured ORP was 714 mV and pH was 6.97 for the chlorine replenishment process (Zhou et al., 2015).

Table 7 presents a comprehensive overview of various parameters influencing water quality and their corresponding impacts. It also outlines the measurement methods for each parameter. The information provided is essential for understanding the significance of these parameters in assessing water quality and designing effective water treatment strategies. Among all the papers selected from the literature search for the data extraction (159 papers), 96 papers studied the impact of temperature on washing operations or on other factors listed above under controlled temperatures, such as ORP or FC. Water temperature determined the activity of oxidant-based disinfectants, FC consumption by organic matter and microbial growth. The higher the temperature (from 10°C to 40°C) the greater log reduction of *Salmonella* due to $ClO_2$ was observed (López-Velasco et al., 2012). However, negative correlation was found between the log count reduction (of ACC and coliforms) and water temperature (Barrera et al., 2012). The concentration of the most active form of chlorine in water, hypochlorous acid, highly depends on the temperature (Randtke, 2010).

Within the retrieved literature, 73 papers studied the impact of pH on washing operations. The active component of chlorine- based sanitisers, hypochlorous acid, predominates at pH 6.0 to 6.5. The pH determines the amount of undissociated (active) chlorine, and therefore the microbial inactivation. High pH values (e.g. > 6.5) of the process water limit the antimicrobial action of disinfectant (chlorine), because the fraction of the most efficient form (hypochlorous) is minimal. pH showed a negative correlation with ORP, indicating a lower oxidant potential of the wash water when the pH was higher. The addition of a weak acid, such as citric acid and/or phosphoric acid, is recommended to improve the stability of chlorine. However, citric acid should be avoided as it leads to the formation of disinfection-by-products (DBPs) (Fan and Sokorai, 2015). Based on the low levels of DBP and emission of chlorine gas, phosphoric acid should be used as pH regulator (Marín et al., 2020). pH control is required when hypochlorite is used, but not with other antimicrobial agents (such as PAA, $ClO_2$ or ozone) (Gombas et al., 2017).

COD is an indirect measurement of organic content, indicating the level of organic and inorganic substances that are accessible to chemical oxidation. The correlation between COD and TOC varies depending on the type and oxidation state of the organic matter (i.e. produce being washed). COD/TOC ratio can vary from 2.5 (Fu et al., 2018) or 3.7 (Weng et al., 2016) for shredded romaine lettuce process water to 8.1 for baby spinach process water (Weng et al., 2016). COD alone may not correlate well to chlorine demand for all types of fresh produce (Chen and Hung, 2016).

Together with total solids, turbidity was identified as the best indicator of organic load (alternative to ORP) (Davidson et al., 2014). The correlation between turbidity and organic load can be impacted by many factors (Gombas et al., 2017). Turbidity is a complex parameter affected by solids content, abundance and solubility of macromolecules such as proteins, the interaction between proteins and phenols, ionic strength of the water and pH relative to the isoelectric point of proteins (Li et al., 2019). Correlation between COD and turbidity, and between COD and UV254 in process water has also been observed before at lab and pilot scale (Luo et al., 2012; Van Haute et al., 2013; López-Gálvez et al., 2019). Mostly due to the increase of COD in process water where PAA is being used, the measurement of turbidity has been recommended for the assessment of the physico-chemical quality of PAA-treated process water (López-Gálvez et al., 2020).

Electrical Conductivity (EC, μS/cm) is the capacity of water to transmit an electrical current and is affected by dissolved solids, which correlated with organic load and should be determined for each type of process water and product (Gombas et al., 2017; Cuevas-Ferrando et al., 2021; López-Gálvez et al., 2021).

UV 254 indicates dissolved organic carbon, while UVA 320 is used as an indicator of suspended solids (Radzevičius et al., 2020). UV254 was correlated with chlorine demand of various types of process water and was selected for further analysis over COD, total protein, total phenolics and turbidity. A study found variations in the relationship between chlorine demand and UV254 among two groups of fruits and vegetables (Chen and Hung, 2016). Group 1 included iceberg lettuce, mushroom, grape, celery, cantaloupe, broccoli and tomato with a steep chlorine demand vs. UV254 correlation slope; Group 2 included romaine lettuce, spinach and strawberry with a flat correlation slope.

Brix° is an indicator of the sugar content (Gombas et al., 2017). This parameter can be considered a good indicator of chlorine demand for certain commodities (e.g. carrot, tomato) but is a poor indicator for leafy greens.

Among the various constituents of produce, amino acids (AA) were observed to be the most likely to react with chlorine, the interaction could be described using second-order reaction kinetics (Abnavi et al., 2021). AA concentration was found as an accurate indicator of chlorine demand in produce wash water along the washing time (Abnavi et al., 2021). PAA was reported in general, to be less sensitive to organic material than chlorine (Zhang et al., 2009).

**Table 7:** Physico-chemical parameters of process water with an impact on the efficacy of the most used disinfection treatments

| Parameter | How to measure? | Impact on water quality |
|---|---|---|
| **Organic and inorganic matter** | Chemical oxygen demand (COD) is a measurement of the capacity of water to consume oxygen during the decomposition of organic matter in the water. | High levels of COD in water indicate the presence of significant amounts of organic matter, which can have several negative effects on water quality, but may not correlate well to chlorine demand for all types of fresh produce. |
| | Turbidity (NTU) is a measurement of the clarity of the water. | High levels of NTU in water can reduce/impair wash water quality, shelter pathogens and increase treatment costs. |
| | Electrical Conductivity (EC, μS/cm) is a measure of the capability of water to pass electrical flow. Dissolved solids affect the capacity of water to transmit an electrical current. | EC in water correlated with organic load, which should be determined for each type of process water and product. |
| | Total Organic Carbon (TOC) is the direct expression of the total organic content. | High TOC can consume chlorine. Solids may protect pathogens against disinfectants. |

| Parameter | How to measure? | Impact on water quality |
|---|---|---|
| | Total soluble solids (TSS) are a measure of the total amount of dissolved solids in water, including salts, minerals and other dissolved organic and inorganic compounds. | TSS in water was identified as one of the best indicators of organic load. |
| | (°Brix) is a measurement of the sugar content and can be used as indicator of soluble organic matter. | High levels of °Brix in water can lead to reduced dissolved oxygen levels, nutrient imbalances, and increased treatment costs. |
| | Total Dissolved Solids (TDS) is a measurement of amount of organic and inorganic materials, such as metals, minerals, salts and ions, dissolved in a particular volume of water. | High levels of TDS in water can lead to increased salinity, nutrient imbalances, corrosion and potability issues. |
| | Maximum Filtrable Volume (MFV, mL) is a measurement of the maximum amount of fluid that can be filtered by a particular filter or filtration system before the filter becomes clogged and requires cleaning or replacement. | MFV can indirectly impact water quality by removing impurities from the water. A high MFV can result in cleaner and safer water, while inadequate filtration can lead to reduced water clarity, harmful contaminants and equipment damage. |
| | Absorbance UV254 (nm) is a measure of the level of organic matter in water, as organic compounds in water can absorb UV light at a wavelength of 254 nm. | High levels of absorbance UV254 in water can indicate the presence of organic matter, including natural organic matter (NOM) from decayed plant and animal material and/or anthropogenic organic pollutants. |
| **Temperature (°C)** | Temperature (°C) can be monitored using on-line systems | Temperature can have a significant impact on sanitation efficacy and microbial growth in water. While higher temperatures can improve sanitation efficacy, they can also promote microbial growth, which can reduce water quality and pose health risks to humans. |
| **pH** | pH can be monitored using on-line systems | pH can have a significant impact on the amount of undissociated (active) chlorine in water and the growth of microorganisms. As pH levels increase, the proportion of undissociated chlorine decreases, which can reduce the effectiveness of chlorine as a disinfectant.<br>Additionally, the use of some biocides and/or acidifiers reduce the pH of the process water contributing to reduce the viability of most microorganisms. |

### 3.9.3. Identification of the most efficacious water disinfection treatments used to maintain the microbiological quality of process water (AQ5/SQ7)

A water disinfection treatment will be considered as 'efficacious' when it is able to maintain the microbiological quality of the process water at a level that avoids microbiological cross-contamination between different batches of ffFVHs during handling and processing operations. To prevent microbiological cross-contamination, the applied disinfectant dose must be able to inactivate pathogens transferred from contaminated produce to the process water almost immediately (time frame within seconds), i.e. before they are transferred from process water to another ffFVH surface.

In static systems ('batch type'), the efficacy of the water disinfection treatment could be measured in terms of inactivation (log reduction) of microbiological indicator or pathogenic organisms in the water and/or the lack of microbiological cross-contamination between different ffFVHs (within and between batches). However, in dynamic systems (e.g. in flume or dump tanks), even if the disinfectant applied to the system inactivates the microorganisms in the water, no apparent reduction may be observed because the microorganisms are being periodically transferred to the water by the regular loading of incoming produce (which may be, albeit unintentionally, contaminated). In this case, the water disinfection treatment applied is considered efficacious when it avoids the accumulation of microbiological indicator or pathogenic organisms in the process water. In practice, under industrial

conditions, the reduction of pathogens is challenging to quantify, due to the inherently low occurrence and levels in fresh produce. Consequently, when assessing the efficacy of the microbiological quality of the process water, microbial indicators are normally quantified. The threshold or critical limit of indicator organisms that defines the proper quality of the process water is dependent on different factors such as the type of processing and handling operations and the type of ffFVH.

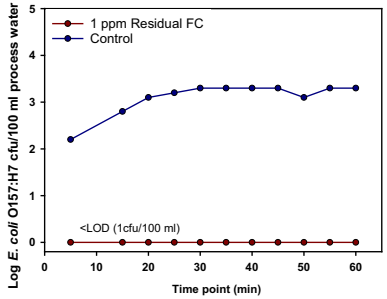
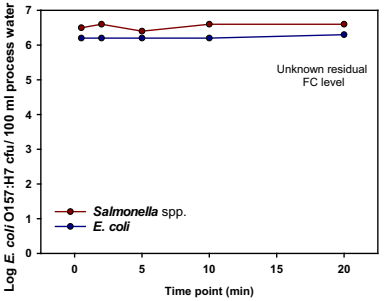
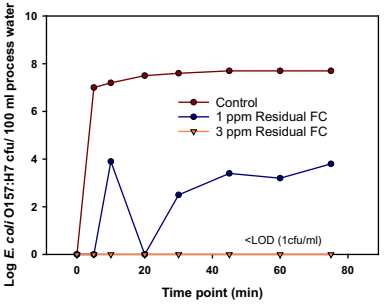
Section 3.9.1 describes the most common disinfection treatments used to maintain the microbiological quality of process water; chlorine-based disinfectants and PAA. These water disinfection treatments have been tested to determine their efficacy. Their efficacy depends on different factors that are usually very specific of the operation, processing line, produce type, treatment type and mode of application, etc. Under lab-scale conditions, all the treatments can be applied under well controlled and optimal conditions, making it relatively easy to be efficacious in controlling the water quality to avoid microbial cross-contamination. However, their efficacy can be drastically reduced when tested under industrial conditions due to the difficulty in achieving the proper conditions of application.

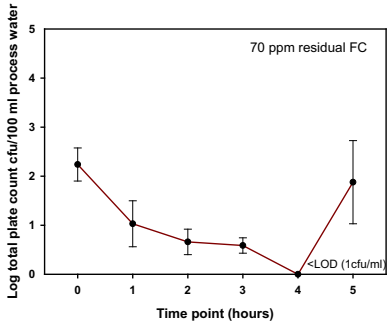
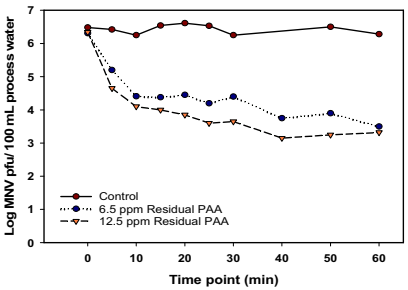
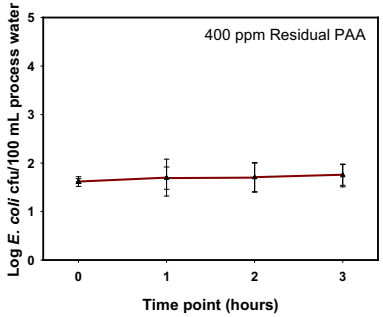
Seven studies have been selected to illustrate how the efficacy of the two most common disinfection treatments (chlorine-based compounds and PAA) can change based on the specific conditions of application during handling and processing operations of ffFVHs.

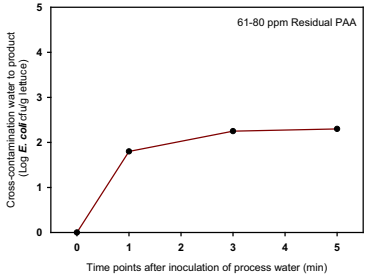
Four studies were selected to illustrate the application of free chlorine to maintain the microbiological quality of process water (Table 8).

- **Study 1** (Van Haute et al., 2013) In this study, experiments were performed at **lab-scale** ('batch system'). The results showed the efficacy of low levels of FC (1.1. mg/L) maintained for 1 h to avoid the accumulation of *E. coli* O157:H7 in the process water even when high COD (1000 mg/L) was present in the process water. Given optimal physico-chemical conditions applied (pH = 6.5 and $T^a < 7°C$), the pathogen was not detected in the treated water, while a gradual accumulation occurred in the control batch without FC application.
- **Study 2** (Banach et al., 2017). This study was also performed at **lab-scale** ('batch system'). An initial FC concentration of 10 mg/L was applied to process water with total organic carbon (TOC) concentrations of 354 and 177 mg/L and to potable water with a TOC of 2.3 mg/L. The pH of the process water was 8.25, which is not appropriate for chlorine-based disinfectants. The residual concentration of chlorine, pH and TOCs were not monitored during the experiment. Cross-contamination of *Salmonella* and *E. coli* during washing of lettuce leaves was observed. It is very probable that the concentration of FC, in the active form (hypochlorous acid), was not enough from the start of the experiment with TOCs of 354 and 177 mg/L, and all the added chlorine was probably consumed by the organic matter present in the process water at the very beginning of the experiment and/or was present in a non-active form due to the non-optimal pH of the process water.
- **Study 3** (Gómez-López et al., 2014) shows the efficacy of chlorine when applied in a **pilot plant-scale** trial ('continuous system'), where the microbial inoculum and organic matter (515 mg/L) were constantly added during the whole duration of the experiment (80 min). In this case, residual FC levels of 3 mg/L maintained during the process was efficacious in avoiding the accumulation of *E. coli* O157:H7 in the process water, while 1 mg/L was not enough. The accumulation of the pathogen in the process water when no FC was present was observed in the control batch. The optimal conditions (pH = 6.5; Temperature = 3°C) applied and constantly monitored ensured the efficacy of the water treatment to avoid the accumulation of the pathogen.
- **Study 4** (López-Gálvez et al., 2019) shows the efficacy of chlorine when applied in an **industrial-scale** consisting of commercial processing line for washing baby leaves. The washing step of the baby leaves line was monitored every hour for up to 5 h and a total of 2,352 kg of product was washed. The COD values changed from 72 $\pm$ 5 to 298 $\pm$ 1 mg/L. The pH of the water was between 7 and 8, and FC levels ranged between 40 and 100 mg/L. Under these conditions, the total coliforms were kept not detected or below 0.5 log CFU/100 mL, while no *E. coli* was detected in any process water sample.

**Table 8:** Studies illustrating the application of free chlorine at lab, pilot plant and industrial scale with various fresh-whole and fresh-cut vegetables and processing conditions

| Study No. | Reference (type of experiment) | ffFVHs | Concentration (mg/L) | Organic matter | Temp (°C) | pH | Target microorganism | Results |
|---|---|---|---|---|---|---|---|---|
| 1 | Van Haute et al. (2013) ("batch trial" at lab-scale) | Butter-head lettuce | 1.1 mg/L residual FC concentration during the trial | COD: 1,000 mg/L | 7 | 6.5 | *E. coli* O157:H7 |  |
| 2 | Banach et al. (2017) ("batch trial" at lab-scale) | Iceberg lettuce | 10 mg/L FC initially dosed (residual FC concentration unknown) | TOC: 354 mg/L | 5 | 8.28 | *Salmonella enterica* subspecies *enterica* serovar Typhimurium and *E. coli* |  |
| 3 | Gómez-López et al. (2014) Pilot plant) | Spinach | 1 and 3 mg/L residual FC concentration during the trial | COD: 500 mg/L | 3 | 6.3–7.1 | *E. coli* O157:H7 |  |

| Study No. | Reference (type of experiment) | ffFVHs | Concentration (mg/L) | Organic matter | Temp (°C) | pH | Target microorganism | Results |
|---|---|---|---|---|---|---|---|---|
| 4 | López-Gálvez et al. (2019) (Industrial scale) | Baby leaves | 70 mg/L residual FC | COD: 275 mg/L | 5.3 | 7.3 | Total plate count |  |
| 5 | Sánchez et al. (2015) (Batch scale) | Romaine lettuce | 6.4 and 12.8 mg/L residual PAA | COD: 500 mg/L | 4 | 7.3 | Murine norovirus (MNV) |  |
| 6 | López-Gálvez et al. (2020) (Industrial conditions) | Bell peppers | 400 mg/L residual PAA | COD > 3,000 mg/L | 25 | 4 | *E. coli* |  |

| Study No. | Reference (type of experiment) | ffFVHs | Concentration (mg/L) | Organic matter | Temp (°C) | pH | Target microorganism | Results |
|---|---|---|---|---|---|---|---|---|
| 7 | Banach et al. (2020) (Industrial conditions) | Fresh-cut lettuce | c.a. 61–80 mg/L residual PAA | TOC: 169.47–210.6 mg/L<br>COD: 446–495 mg/L | 3.0–3.9 | 6.8–6.9 | *E. coli* |  |

Three studies have been selected for the application of PAA at laboratory and industrial scales (Table 8):

- **Study 5** (Sánchez et al., 2015) shows **a lab-scale experiment** ("batch system") in which low concentrations of PAA (< 13 mg/L) were applied to process water (COD = 500 mg/L) that had been previously inoculated with murine norovirus ($10^4$–$10^7$ $TCID_{50}$/mL). In this batch study, the initial inoculum applied to the process water was reduced by the application of PAA with a contact time of 60 min, but no constant addition of organic matter or inoculum was applied, which differs of what occurs in the real industrial conditions.
- **Study 6** represents **an industrial application** of PAA in a washing tank of whole bell peppers (López-Gálvez et al., 2020). This scenario illustrates a real application of PAA in a packinghouse. PAA was applied at a high concentration (> 400 ppm) and the process water contained a high concentration of organic matter (COD > 3,000 mg/L) mostly due to large amounts of product washed in the same process water (1,000 L). In this case, the applied water disinfection treatment was not able to prevent the detection of *E. coli* in the process water, and constant levels (1.5–2 log CFU/100 mL) were observed during the 2 h of the experiment. This example illustrates the reduced efficacy of this PAA treatment under industrial conditions.
- **Study 7** (Banach et al., 2020) reflects an **industrial application** of a PAA solution during the washing of fresh-cut lettuce. The PAA solution was applied at a target concentration of 75 mg/L directly to a single wash tank (3,500 L) during about 90 min of washing of fresh-cut lettuce (c.a. 800 kg). The ratio of produce to water was 0.23 kg/L. The *E. coli* inoculum ($10^6$ CFU/mL) was added to the process water after 90 min of the running of the processing line. When non-inoculated fresh-cut lettuces were washed in the inoculated process water (treated with PAA), washed lettuce showed between 2 and 3 log reductions of *E. coli*. These results demonstrate that the applied PAA-based disinfection treatment was not efficacious to avoid cross-contamination of lettuce during washing at industrial scale.

The use of water disinfectants during processing and handling operations, and the effects on the microbiological cross-contamination, would require further research to demonstrate their efficacy in real industrial settings and commercial operating conditions.

Based on these illustrative examples, among these two most common water disinfection treatments, chlorine has been reported to be an efficacious treatment provided that is applied under appropriate (optimal) conditions, as it is able to avoid the accumulation of microorganisms under the conditions in the fresh produce industry (Table 9). Most of the scientific evidence relates to the efficacy of chlorine-based water disinfection treatments against bacterial cells. However, the use of any water disinfection treatment should follow good water management strategies, respecting the physico-chemical parameters and process parameters, to support the efficacy of water disinfection treatments.

### 3.9.4. Impact of different water disinfection treatments on the induction of the viable but non-culturable (VBNC) state (AQ6/SQ8)

Several studies have provided evidence that many bacterial species, including food-borne pathogens, when subjected to specific stresses, are able to develop resistance mechanisms that enable them to enter a temporary state of low metabolic activity in which cells can persist for extended periods without division, called dormancy. Two dormancy states have been described in non-sporulating bacteria: viable but non-culturable (VBNC) cells and persistent cells. Although they share some similarities, VBNC cells do not divide, but they maintain their intact cells membranes, low metabolic activity, continued gene expression and can become culturable once resuscitated. However, they are unable to immediately recuperate the ability to divide when plated on a laboratory medium (Zhao et al., 2017). Most bacterial species enter the VBNC state in the presence of adverse environmental conditions, including exposure to biocides commonly used for water disinfection (chemical disinfectants), indicating that dormancy might be the default mode in the bacteria life cycle.

On the other hand, most persistent cells are slow or non-growing sub-populations that might exhibit multidrug tolerance and survive antimicrobial treatments while the rest of the population is sensitive (Ayrapetyan et al., 2015b). This antimicrobial tolerance is associated with physiological changes that may be stochastic or environmentally induced as opposed to mutational events leading to non-reversible resistance (Ayrapetyan et al., 2018). In fact, exposure of bacterial cells to sub-lethal stressors may induce both, sublethal injury (persistent cells) and the VBNC state (Arvaniti et al., 2021).

Several authors support the hypothesis that persistence and VBNC cells are related and part of a 'dormancy continuum', in which they share similar mechanisms but are found in different physiological positions on the dormancy range (Ayrapetyan et al., 2015a). Ayrapetyan et al. (2015b) demonstrated that VBNC cells are present during persistent cell isolation experiments, further indicating that these cells coexist and are induced by the same conditions. (Mu et al., 2021) support the hypothesis that the changes in the cell state represent a continuum between cells that are actively growing and dead cells with VBNC cells being in a deeper state of dormancy than persistent cells. In fact, Ayrapetyan et al. (2018) explained that in many species, dormancy is not necessarily an on/off switch but rather a progressive, stepwise process that changes dynamically with time and can be stochastic or a result of environmental cues, supporting the current evidence that the VBNC state and persistence may be physiologically related and part of a continuum of dormancy.

Sub lethally injured cells are inhibited by selective agars but may grow on non-selective media (Li et al., 2014; Schottroff et al., 2018). Persistent cells are induced by the exposure to adverse environmental conditions, which reversibly damage the cell structures and reduce the cell functions. However, severely injured cells that cannot be resuscitated under appropriate conditions may enter the VBNC state (Wesche et al., 2009; Schottroff et al., 2018). The fact that injured cells cannot form colonies on selective media, whereas non-selective media enable the recovery and growth of the organisms, can be used to differentiate individual physiological states (stressed vs. non-stressed cells) (Kell et al., 1998; Colwell, 2009).

Standard plate count procedures are usually applied to determine the rate of bacterial inactivation. Some studies have reported discrepancies between the levels of *E. coli* quantified by cultivation-based techniques and molecular techniques in combination with dyes such as the PMA-qPCR assay (Moyne et al., 2013; Gensberger et al., 2014; Truchado et al., 2016). One characteristic of sublethally injured and VBNC cells is that they may evade detection, resulting in underestimation of a food product's microbial load (Arvaniti et al., 2021). Induction of the VBNC state is crucial for food-borne pathogens, such as *L. monocytogenes*, the detection of which relies almost exclusively on the use of culture recovery techniques. The use of culture recovery techniques (e.g. plating) may provide false-negative results of the inactivation capacity, consequently resulting in an overestimation of the inactivation treatment.

There are numerous and different methods developed for the quantification of VBNC cells however, all existing methods have limitations (Arvaniti et al., 2021). Sub-lethally injured bacteria are typically analysed by differential enumeration using non-selective and selective growth media while methods used for the detection of bacterial viability are mostly based on the measurement of cellular integrity, metabolic activity, detection of respiration or presence of nucleic acids (Schottroff et al., 2018).

The **combination of dyes** and **flow cytometry** has been widely used to determine the cell viability of food-borne pathogenic bacteria (Léonard et al., 2016), but it is not suitable for all matrixes. Instead, viability quantitative polymerase chain reaction (v-qPCR) has been widely adopted to detect and quantify the presence of viable bacteria in specific food matrices and water (Truchado et al., 2016; Dorn-In et al., 2019). These techniques are based on the cell membrane integrity to differentiate between dead and VBNC cells, if dead cells have the membrane damaged while VBNC and viable cells have an intact membrane (Oliver, 2010). However, as not all the dead cells have their cell membrane compromised, these methods can lead to an overestimation in the number of VBNC cells. To solve these problems, the qPCR methodology has been combined with the use of two photoreactive dyes such as propidium monoazide (PMA) and ethidium monoazide (EMA) (Figure 10). PMA is a DNA-dye, which allows the differentiation between viable and dead cells, avoiding an overestimation of results by qPCR. This technique is based on the ability of PMA to penetrate the dead cells with compromised membrane integrity and bind covalently to the DNA and free-DNA after photoactivation, thus preventing subsequent PCR amplification (Nocker and Camper, 2006). However, this dye is only able to attach to the bacterial DNA when the membrane is compromised. On the other hand, EMA can diffuse across cell membranes using efflux pumps (Codony et al., 2015). 'EMA in contrast to PMA can also penetrate viable cells of some bacterial species due to a lower charge. Metabolically active cells export EMA via transport pumps actively or passively via diffusion barriers out of the cell. Nevertheless, the remaining chemical residues in viable cells lead to a substantial loss of DNA resulting in false-negative results' (Fleischmann et al., 2021).

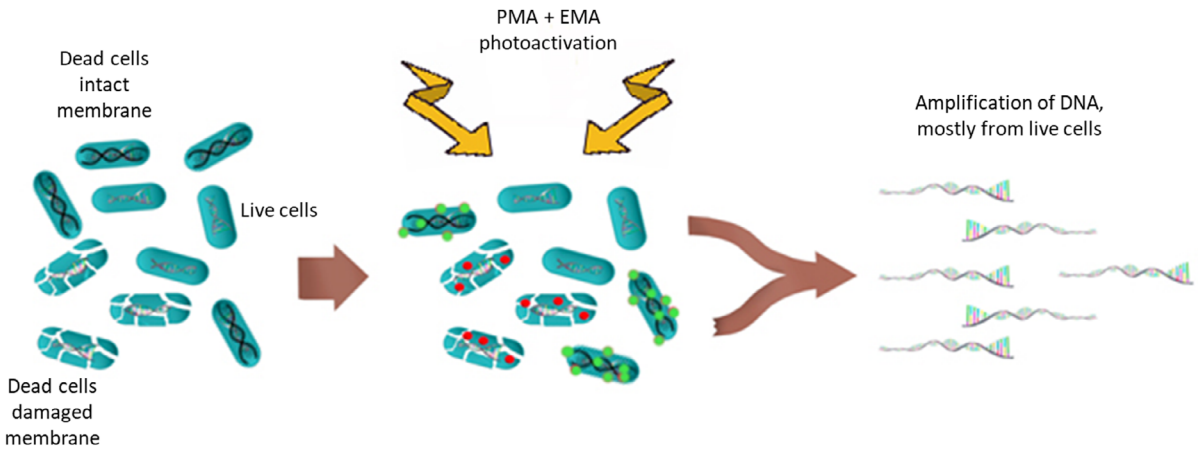



**Figure 10:** Schematic illustration of the PMA and EMA use to avoid amplification of DNA coming from dead cells

**Other techniques** have been applied to the detection of VBNC cells, including the direct fluorescent antibody–direct viable count (DFA–DVC) method, substrate responsiveness combined with fluorescent in situ hybridisation (DVC–FISH assay) and LIVE/DEAD BacLight bacteria viability kit combined with flow cytometry (Lv et al., 2020). The direct viable count (DVC) is one of the most popular and widely used methods of detecting viable cells in environmental samples. The methods recognise the viable cells because of enlarged, elongated morphology. The DFA-DVC method has been applied to detect very small numbers of organisms in food and water samples by concentration of bacteria on filters prior to staining (Hasan et al., 1995). However, most of these methods are still more expensive than v-qPCR, and in many cases technically challenging, or unable to conduct quantification in process water. More recently a novel strategy has been developed through direct metatranscriptome RNA-seq and multiplex RT-PCR amplicon sequencing on Nanopore MinION. This technique seems to be a promising method to achieve real-time multiplex identification of viable pathogens in food (Yang et al., 2020). These authors demonstrated that direct RNA-seq and RT-PCR amplicon sequencing of the metatranscriptome, enabled the direct identification of nucleotide analogs in RNAs.

In the current SO, the induction of persistence and VBNC state in bacterial cells is considered because of the presence of adverse environmental conditions when using water disinfection treatments to maintain the microbiological quality of process water. The mechanisms of action of each water disinfection treatment highly affects the induction of sub lethally injured cells or VBNC.

Sublethal injury of microorganisms implies damage to structures within the cells, the expression of which entails some loss of cell function that may be transient or permanent (Gilbert and Pettigrew, 1984). Optimal residual concentrations of water disinfectants in the washing tank must be set at a level above the minimum concentration needed to maintain the microbiological quality of the process water. For chlorine, several authors have suggested that 10 mg/L of free chlorine (FC) could be the lowest effective concentration for most leafy greens (Gombas et al., 2017; Luo et al., 2018), while other authors demonstrated that higher concentrations up to 20–25 mg/L of FC were needed (Tudela et al., 2019b). Under these conditions, it has been demonstrated that bacterial species enter the persistence and VBNC states (Arvaniti et al., 2021; Truchado et al., 2021). Therefore, the use of conventional plate count methods might lead to an overestimation of the efficacy of biocides and, consequently, to the failure to detect a food safety issue (Truchado et al., 2020).

Truchado et al. (2021) demonstrated that the recommended performance standard for sodium hypochlorite (20–25 mg/L free chlorine) was effective in inactivating *L. monocytogenes* and *E. coli* O157:H7 in the different process water. However, recommended concentrations of peroxyacetic acid (PAA, 80 mg/L) and chlorine dioxide ($ClO_2$, 3 mg/L) reduced the levels of culturable pathogenic bacteria but induced the VBNC state of the remaining cells. Similarly, Arvaniti et al. (2021) showed that PAA had a milder effect than sodium hypochlorite, inducing sublethal injury and it also allowed cellular recovery following 1 min of exposure to 40 mg/L. This is also consistent with the observations by Gu et al. (2020), who reported that incubation for 30 s in 30 mg/L PAA resulted in undetectable population levels of *L. monocytogenes* by selective plating. Having the majority of human disease genes and disease pathways, *Caenorhabditis elegans* has emerged as a promising model for the assessment of virulence of numerous human pathogens (Kaletta and Hengartner, 2006). This model

has been used to determine the potential pathogenicity of VBNC cells in chlorine-induced VBNC *L. monocytogenes* and *Salmonella* Thompson. The ingestion of VBNC cells by the worm considerably reduced its life span. In fact, no significant difference between the life span reductions caused by the VBNC and culturable pathogenic cells was observed, emphasising the risk that VBNC food-borne pathogens could pose to public health (Highmore et al., 2018). However, the result can only be considered as indicative and more evidence is required to determine whether or not VBNC *L. monocytogenes* and *Salmonella* Thompson are infectious to humans by resuscitation or in the VBNC state. Therefore, the relationship between these results and risk to humans have not been demonstrated.

### 3.9.5. Impact of different water disinfection treatments on the induction of VBNC to recover and/or express virulence in ffFVHs (AQ6/SQ9)

Resuscitation of VBNC bacterial cells has been described in some bacterial species including *E. coli* O157:H7 and *L. monocytogenes* and it has been defined as the capability of VBNC cells to recover their metabolic activity and culturability (Li et al., 2014; Wei and Zhao, 2018; Dong et al., 2020). As far as we know, few studies have been focused on the capability of resuscitation of VBNC bacterial cells present in disinfected water and those available have observed contradictory results (Afari et al., 2019; Gu et al., 2020). Resuscitation of VBNC cells has been achieved using different methods, such as the removal of stresses and nutrient supplementation (Dong et al., 2020). However, resuscitation of VBNC cells has not been always observed in different studies, probably due to the application of different experimental conditions or resuscitation window (Dreux et al., 2007; Dinu and Bach, 2011; Van Der Linden et al., 2014; Dong et al., 2020). As far as we know, there have been some minor attempts to demonstrate the capability of resuscitation of VBNC bacterial cells present in fresh produce and the results are often contradictory. In 2007, Dreux et al. investigated the presence of VBNC *L. monocytogenes* during survival and recovery to VC state on parsley leaves. These authors concluded that dry conditions induced VBNC *L. monocytogenes* in parsley leaves but these cells were unable to recover sufficiently to be culturable after transfer to wet conditions. However, as stated by Li et al. (2014), the major obstacle that researchers encounter when performing resuscitation studies is the difficulty to differentiate between the resuscitation of VBNC cells and the normal growth of residual culturable cells. Dinu and Bach (2011) stated that metabolically active VBNC *E. coli* O157:H7 induced by low temperature on the phyllosphere of lettuce could produce small amounts of Shiga toxin, highlighting the importance of monitoring VBNC cells of human pathogens that may be infectious and pose a potential health risk. Lately, Van Der Linden et al. (2014) reported that stressed *E. coli* O157:H7 cells inoculated on lettuce leaves were able to recover and attach to the lettuce surface. They also indicated that no significant differences in attachment were observed between stressed and freshly cultured cells.

Truchado et al. (2023) demonstrated the capacity of *L. monocytogenes* and *E. coli* O157:H7 VBNC cells to cross-contaminate shredded lettuce during washing and their ability to survive and resuscitate during the shelf-life of the product. Based on these results, it could be concluded that *L. monocytogenes* VBNC cells present in process water were able to be transferred to shredded lettuce during 1 min washing. However, under commercial storage conditions, the probability of resuscitation from the VBNC state to a culturable state and potential growth during storage of the fresh produce was very low.

### 3.9.6. Main remarks

Section 3.9.1 (AQ5-SQ5)

- Studies on water disinfection treatments used during post-harvest operations of fresh-whole or fresh-cut fruits and vegetables were found through the literature search, while studies on fresh or frozen herbs are limited. Conclusions on common water disinfection treatments for herbs can at most be assumed to be similar to those of fruits and vegetables.
- According to the available literature, water disinfection treatments are chemical, physical, biologically based or combinations thereof. Their industrial-scale application during post-harvest operations of ffFVHs is scarcely reported in the scientific literature, with available literature noting that chemical-based water disinfection treatments are more often reported.
- At an industrial scale in some EU countries, chlorine-based disinfectants and PAA are used as water disinfection treatments for fresh-whole and fresh-cut fruits and vegetables.

- Alternative disinfectants, including the combination of chemical and physical technologies, are upcoming, yet would require further research on, i.e. their safety, costs and consumer acceptance before application.
- Overall, chlorine-based disinfectants and PAA have been reported in the literature and industrial practices as common water disinfection treatments used to maintain the microbiological quality of process water of ffFVHs.

Section 3.9.2 (AQ5/SQ6)

- Most studies showed that the disinfection efficacy is closely associated with physico-chemical parameters of process water.
- The physico-chemical parameters of process water with an impact on the efficacy of the most used disinfection treatments are: (1) organic and inorganic matter present in the process water; (2) pH; and/or (3) temperature.
- Many different parameters can be used to determine the concentration of organic and inorganic matter in the water such as: chemical oxygen demand (COD), turbidity (NTU), electrical conductivity (EC), total organic carbon (TOC), total suspended solids (TSS) or Brix, total dissolved solids (TDS), Maximum Filtrable Volume (MFV, mL) or UV 254.
- Most studies focused on how pH and COD affect efficacy of chlorine, however, optimal conditions for other water disinfection treatments have not been determined.

Section 3.9.3 (AQ5/SQ7)

- A water disinfection treatment will be considered as 'efficacious' when it is able to maintain the microbiological quality of the process water to a level that avoids cross-contamination with pathogens during the handling and processing operations.
- Based on the literature, the common water disinfection treatments applied to process water have been shown to be capable of avoiding the increase of microbial load and even reduce it under lab-scale trials and optimum conditions. However, there is a dearth of published studies on the efficacy under real industrial conditions for most of the processes and ffFVHs.
- There is not one specific water disinfection treatment that can be applied to all the handling and processing post-harvest operations under any condition. The efficacy of water disinfection treatments depends on the physico-chemical parameters and conditions that are applied.
- Among the common water disinfection treatments, chlorine-based biocides have demonstrated the capacity to avoid accumulation of microorganisms in process water under industrial conditions. However, their application should be properly managed to obtain the best performance (e.g. FC, pH, temperature, organic matter, etc.).
- For other disinfection treatments (e.g. PAA), other parameters are important. However, studies showing efficacy of PAA to prevent cross-contamination of produce during handling and processing operations under real industrial conditions were not found.

Sections 3.9.4 and 3.9.5 (AQ6/SQ8 + AQ6/SQ9)

- The two dormancy states described in non-sporulating bacteria are the viable but non-culturable (VBNC) cells and persistent cells.
- Persistent cells are in the initial stages of dormancy, with quick resuscitation potential upon the removal of the stress conditions, while VBNC cells are in a deeper state of dormancy.
- There are numerous different methods developed for the quantification of VBNC cells. However, all existing methods have limitations. Determining the viability of non-culturable cells is mostly based on the measurement of cellular integrity, metabolic activity, detection of respiration or presence of nucleic acids.
- The mechanisms of action of each water disinfection treatment as well as the concentration and contact times highly affect the induction of sublethal injured or VBNC cells of pathogenic bacteria.
- The potential pathogenicity of VBNC cells has been demonstrated in chlorine-induced VBNC *L. monocytogenes* and *Salmonella* Thompson using *C. elegans* as a model. However, the results of this study alone cannot be considered as absolute proof that VBNC pathogens are or are not infectious to humans by resuscitation or in the VBNC state.
- *L. monocytogenes* and *E. coli* O157:H7 VBNC cells were shown to be able to cross-contaminate shredded lettuce during washing and resuscitate during the shelf-life of the product.

## 3.10. Efficacious water replenishment rate to maintain the appropriate microbiological quality requirements of water (AQ7)

The frequency and rate of water replenishment contributes to dilute the organic matter and the level of microorganisms present in the process water and should be determined by the industry for each specific case (Holvoet et al., 2012). If the concentration of organic matter present in the process water reached a specific threshold, water should be replenished to maintain a specific and optimal residual concentration of biocides and then avoid the accumulation of microorganisms. Each processing line will have a specific threshold of COD, turbidity and TSS, up to which the quality of the water cannot be maintained by the sole addition of biocides (see Section 3.8.3 on efficacious water disinfection). Therefore, apart from an efficient application of biocides, water replenishment will be a critical activity in water management.

From the industry survey, respondents reported that various water disinfectant-to-water replenishment strategies are implemented. Some FVH processors did not report the amount of water replenished during processing with water disinfectants, either because the data was unknown or unavailable, while others reported in $m^3$ or litres per hour. For instance, in processes with sodium hypochlorite, water was reported to be replenished at 0.5–1 $m^3$/h or, more generally, 'continuous', as was the case of rinsing water used to make fresh-cut salads. In comparison, washing diced/sliced onions with no water disinfectants had a water replenishment rate of 4 $m^3$/h, while for washing spinach with no water disinfectant, 5 $m^3$/h was applied. Respondents more frequently reported when they had a complete water change. This ranged from no replenishment to continuous replenishment. Also practiced was water refreshment after every few hours or days to after 1 or 2 weeks, depending on the product type or amount of product processed.

Water replenishment rates of 1.3 $m^3$/h have been proven to be inefficient to maintain the microbiological quality of process water in a fresh-cut iceberg processing plant, while water replenishment rates closer to 10 $m^3$/h have been recommended (Allende et al., 2008a). However, this high-water demand, could be difficult to implement due to the elevated cost of the water and the water scarcity in some regions across Europe. Also, in the frame of a sustainability assessment, more and more FBOps are investigating if water can be reused both with and without treatment. The recirculation of the water used in the different post-harvest handling and processing operations is a key consideration for the sustainability of the system (Gil et al., 2009).

Some water management systems rely only on the use of very high-water replenishment rates in the washing tank to avoid the accumulation of microorganisms in the process water, without the combination of a biocide. In these cases, the ideal situation would be to use a shower (spraying of water) instead of a washing tank (immersion in water), to avoid any risk of cross-contamination. However, these strategies demand the use of very large volumes of water, which, as mentioned before, are not compatible with environmentally sustainable practices. Alternatively, these high-water replenishment rates could be combined with an offline water treatment based on, for instance, reverse osmosis to bring the effluent process water again to a fit for use or clean water level before reintroduction in the wash tanks or processing lines. Other water management strategies (McEntire et al., 2016) developed and patented a single-pass commercial system that uses retreated, spent wash water (solids and organics removed to produce clean water) in a series of over-head sprayers. This system sprays sanitised water onto fresh-cut produce in a single-pass to avoid the reuse of process water with accumulated organic load. The system is also designed to tumble the produce so both sides are exposed to the chlorinated wash water. The spent wash water is collected at an onsite water treatment facility and the recycled treated water is reused to wash produce.

### 3.10.1. Main remarks

- During the washing process, soils, plant debris and other organic materials released from cut or damaged produce continue to accumulate in the water while biocides are inactivated rapidly, and thus consequent loss of efficacy has been shown to be problematic.
- To avoid accumulation of microorganisms in the process water, water should be replenished sufficiently often to maintain a specific and optimal residual concentration of biocides.
- Each processing line will have a specific threshold of COD, turbidity and TSS, up to which the quality of the water cannot be maintained by the sole addition of biocides.
- In case the water management is solely based on water replenishment, and not combined with the application of a disinfectant, high volumes of fresh clean water are needed to avoid cross-

contamination in the processing lines. In this situation, the use of showers or sprayers in combination with offline water treatment are needed to avoid cross-contamination and to reduce the use of high volumes of water.

- When biocides are applied as part of the water management strategy, replenishment of water will be needed to dilute the accumulation of organic material, which are inhibiting the efficacious activity of the active compound of the biocide.
- There is a lack of research papers focused on process water replenishment under controlled conditions, especially the effect of replenishment rate on the microbiological quality of process water. Therefore, the information from the retrieved literature is limited for future application in industrial scale.
- The replenishment rate and water use retrieved from the industrial survey were mostly estimated or calculated indirectly. The accuracy of information needs to be verified further. The current information about the impact of water replenishment rate is very uncertain as there are only very few studies considering water replenishment as an intervention strategy.
- No information is currently available in the literature related to suitable combinations of water disinfection and water replenishment rates.
- Data obtained from the outsourcing activity linked to this mandate will be analysed to provide recommendations on water management practices for three different sectors (fresh-whole, fresh-cut and frozen FVHs). This information will be presented in future opinions.

## 3.11. Relevant protocols (including parameters, analytical methods and frequency) to validate and/or verify the appropriate microbiological quality requirements of the water intended to be used for different post-harvest handling and processing operations of ffFVHs (AQ8)

The control measures, such as water management strategies and water replenishment, which are included in the water management plan of a FBOp to achieve and maintain the microbiological quality of water used in processing operations, need to be validated, monitored and verified as summarised in Table 9.

Microbiological testing for the occurrence of pathogens in end products (e.g. packed and shredded fresh-cut vegetables or other ffFVHs) is rarely useful as verification that cross-contamination in the production process is avoided. Due to the low occurrence of pathogens in the raw materials and the further dilution of the pathogens in water, an intensive sampling regime and very sensitive testing methods would be needed to detect the presence of a pathogen. If the pathogens are present below the limits of detection an apparent 'negative' result may give a false sense of safety to the FBOp. Therefore, as stated in Section 3.9.3, it is recommended to use indicator organisms in combination with the monitoring of the process parameters of the water management strategies for the effective implementation of the applied water management in the diverse post-harvest handling and processing operations.

**Table 9:** Overview of validation, operational monitoring and verification within the water management system in post-harvest handling and processing operations of ffFVHs

| | Validation | Operational monitoring[(a)] | Verification |
|---|---|---|---|
| **Definition (Commission Notice 2022/C 355/01)** | Obtaining evidence that a control measure or combination of control measures (water disinfection and/or replenishment), if properly implemented in the HACCP-based procedures and by the OPRP, can control the hazard to a specified outcome. | The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure (water disinfection and/or replenishment) is under control. | The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended. Verification is conducted periodically to demonstrate that the HACCP system and the management of the OPRPs are working as planned. |

| | Validation | Operational monitoring[(a)] | Verification |
|---|---|---|---|
| **Aim** | To set critical limits of relevant defined parameters influencing the efficacity of the water treatment in the process e.g. concentration of biocide, physico-chemical parameters, etc. that assure that the target microbiological inactivation is achieved or the cross-contamination via the process water is avoided, considering the reasonably foreseeable variability of operating conditions. | To control if the defined (physico-chemical) parameters are within the validated critical limits to follow-up if the operation (production process) is running as intended and in case of deviation, immediate action can be taken to correct the process. | To check if the microbiological quality of process water is achieved by the implementation of the operational conditions that are being monitored. To follow-up the validated production operation/process to check the compliance of the performance standards regarding microbiological target organisms in process water. |
| **When** | A priori during setting-up processing conditions | Real time during the operation/process | A posteriori, after the operation/process has been carried out |
| **Frequency** | In case of changes in the processing conditions (i.e. process conditions such as water/product ratio, change in equipment and tools etc.). | During each processing batch. Providing results with sufficient frequency that enables identifying failures in a timely manner to allow a rapid response (i.e. corrective action to be taken before use of the water supply). | Periodically, depending on the water management strategy and stability of the process. When historical information is built up by the FBOp, the frequency can be reduced. |
| **What** | Indicator microorganisms, - residual concentration of disinfectant as well as relevant Physico-chemical parameters (e.g. pH). | Mainly physico-chemical parameters that can be measured in real-time (e.g. FC, pH). | Indicator microorganisms Physico-chemical parameters Records of operational monitoring Instrument calibration results. |
| **How** | Tailored procedures carried out in-plant, with the actual process equipment, covering realistic boundaries of reasonably foreseeable operating conditions. | Ideally through continuous at/on/in-line systems providing real time information on the status of the physiochemical parameters or concentration of biocide. Alternatively, off-line measurements through rapid analytical methods. | Trend observation or trend analysis of reviews and checks of the records of the operational monitoring and status of calibration of instruments. Off-line measurements of parameters of the process water e.g. indicator organisms, physico-chemical parameters. Results review and trend observation/ analysis. |

(a): The term 'monitoring' is often used in scientific literature to refer to several purposes, including verification and validation. The term 'operational monitoring' is used in the FAO guidance (FAO and WHO, 2021a,b), which may help to specifically refer to the real-time measurements of process control parameters.

This section is based on the concepts and principles of several documents that specifically deal with the approaches for validating, monitoring and verifying control measures aiming to eliminate pathogenic microorganisms and/or prevent re/cross-contamination, either as general guidelines in the frame of HACCP (NACMCF, 2010; ICMSF, 2011b,d,e; Bracket et al., 2014) as well as in CAC 2008[15] and

[15] CAC (Codex Alimentarius), 2008. Guidelines for the validation of food safety control measures. CAC/GL 69–2008. p. 1–16.

ISO 20976-2:2022[16]) or more specifically referred to process water and/or produce washing operations (Holvoet et al., 2012; Gombas et al., 2017; FAO and WHO, 2021a).

### 3.11.1. Relevant protocols for validation

**Validation** is defined as the procedure that provides the scientific and technical evidence that a control measure, when properly implemented, can control the identified hazard to a specified outcome, i.e. no accumulation of microorganisms (CAC, 2008[15]; Gombas et al., 2017; European Commission, 2022[10]).

In the context of process water for ffFVHs, the goal of the validation is to obtain evidence that the required microbiological quality of the process water is achievable and thus cross-contamination during the handling and processing operations is avoided. Validation procedures allow definition of the appropriate operational criteria associated with water management strategies (e.g. critical limits of water disinfection and/or replenishment related to certain physico-chemical parameters of the process water) allowing to control the relevant microbiological contamination (FAO and WHO, 2021a). The industrial operations and associated conditions using process water are very specific for the product, production line, facility, industry, country, etc. Therefore, validation procedures must be tailored to the uniqueness of each process line and water management strategy existing in a facility. To obtain robust and reliable results, validation should be carried out in-plant using the actual process equipment of the specific facility, under realistic conditions. The dynamic nature of the process and changes of microbiological load in water over time must be considered together with the reasonably foreseeable variability of the operating factors and parameters influencing the performance of the processing operation (Davidson et al., 2014; Gombas et al., 2017). In this respect, studies carried out at laboratory and pilot-plant scale are of limited value.

For validating the operation process associated with FVHs washing or post-harvest use of water, several steps are generally needed (Figure 11), which are described below.


**Figure 11:** Steps of the validation of the standard operation procedures associated with use of water in post-harvest process of ffFVHs

**[1] Defining the aim and scope of the validation**

First, the specific aim of the validation should be defined. Different purposes can be pursued when planning the validation of a ffFVH handling or processing operation involving process water (i.e. water replenishment rate and/or disinfection treatment). The validation, under reasonably foreseeable operating conditions will aim to demonstrate that cross-contamination can be prevented by maintaining the microbiological quality of process water including providing information on:

- the minimum dose of water disinfection treatment needed to control the microbiological quality of the process water is achieved and maintained and/or
- the volume of water replenishment during processing in a certain time frame avoids accumulation of microorganisms in the process water;

Validation procedures may include other more specific purposes, such as mapping the washing equipment to identify the worst-case locations (e.g. showing lowest concentration/dose of disinfectant) where the sensors for key monitoring parameters should be placed (Gombas et al., 2017).

The scope of the specific processing operation must be identified. Separate validations might be required for multistage systems consisting of more than one flume or wash tank, i.e. when independent operating conditions are placed (e.g. disinfectant dosage, replenishment rate, recirculation system, etc.).

[16] ISO 20976-2:2022. Microbiology of the food chain – Requirements and guidelines for conducting challenge tests of food and feed products – Part 2: Challenge tests to study inactivation potential and kinetic parameters. International Organization for Standardization. Geneva. Switzerland.

### [2] Defining the operation conditions of the process/es

The robustness and variability of the operating conditions of the process to be validated must be characterised. This step requires a considerable effort for the collection of relevant information of the water quality management practices, the critical factors affecting the performance of the process and limits of the conditions within which the FBOp intends to operate.

The aim is to define the reasonably foreseeable scenarios that need to be considered in the validation. The range of conditions should cover the operating extremes which are still acceptable within the operating boundaries (some guidelines term it as 'worst-case', Gombas et al., 2017).

Some of the process parameters will **remain constant (fixed)** in every processed batch, for instance equipment dimensions, type of disinfectant, water source, water filtration etc., which need to be recorded and reported. If any of these parameters change, it will require consideration as to whether the process is still operating within the originally validated process. A re-validation will be needed in that case.

The conditions that can be **variable** between batches and/or during a process (e.g. during the production day or week) need to be characterised. The boundaries of the reasonably foreseeable operating conditions described below, and which should be considered in the validation have to be defined. The actual conditions occurring during the validation will be monitored and recorded (some of these parameters will become critical limits of acceptability).

Some of the variable key factors in a process may include:

- **Product related factors**: type(s) of commodity, mode of cut (e.g. chop, shred and cut), product feed rate, amount of dust/soil/organic matter in the ffFVHs, etc. For lines processing multiple commodities the validation might be carried out for that providing the worst-case scenario. However, differences between commodities may require separate validations to avoid excessive processing (e.g. hyperchlorination).
- **Water related factors:** water hardness, water temperature, water flow, refreshment or replenishment rate, the product-to-water ratio, turbulence, etc. The dynamic nature of relevant physico-chemical parameters (pH, organic matter and mineral load, solids level, etc.) and the microbial load in the process water must be considered.
- **Water disinfection treatment related factors:** type (commercial product), concentration, mode of application, disinfectant feed rate, etc. The knowledge of the chemistry of the disinfectant and its active form is key. Besides the level of disinfectant and the dosing regime, the temporal and spatial changes of the residual concentration of the disinfectant (in the active form) and the physico-chemical composition of the process water that affect the disinfectant efficacy must be addressed, making sure that the reasonably foreseeable variability is covered.

Preliminary assays may be needed either (i) to identify the locations of the system that correspond to the extremes of the variability of the conditions (e.g. with the lowest concentration of disinfectant or time of the day with the highest organic load in the water) and/or (ii) to adjust the conditions of the water treatment (replenishment rate, concentration of disinfectant, etc.) to be validated. When available, the use of decision support tools based on predictive models (see following opinions) can be used for this purpose.

### [3] Target microorganism/s and performance standards

#### Target organisms

Regarding the target microorganism/s to validate a water treatment, rather than testing for pathogens (which rarely occur at detectable or quantifiable levels), in-plant validation procedures should focus on microbial indicators. As reviewed in FAO and WHO (2021a), despite being no single organism that fulfils all the required properties for the ideal indicator, *E. coli* is often used as an indicator to assess the microbial quality of process water and as an indicator of the behaviour of enteric pathogens such as *Salmonella* spp. or pathogenic *E. coli* into the water management system. The statistical correlation between faecal indicators and pathogens is hardly observed in low contaminated water. However, the higher the faecal contamination (represented by *E. coli*), the higher the likelihood that the presence of enteric pathogens is established in preharvest conditions (e.g. soil, manure, irrigation water) (Ceuppens et al., 2015; Truchado et al., 2018). Most recommendations on contamination levels in water used in post-harvest activities when the product is in contact with process water indicate that potable water should be applied for final washing (FAO and WHO, 2019; European Commission, 2017[3]). However, in some cases flexibility is given for non-ready to eat FVHs

and process water used for the first washing of product, in case of RTE FVHs (100 *E. coli* cfu/100 mL) (European Commission, 2017[3]). Holvoet et al. (2012) observed that *E. coli* levels higher than 2 log CFU/g on the processed lettuce were measured when numbers of 5 log CFU/100 mL were observed in the process water. However, lower numbers in the water does not imply the 'absence' of *E. coli* on the lettuce, as no enrichments were conducted in the study, and enumeration results only above the detection limit results could be stated. Similar contamination results were obtained for *E. coli* O157 and viruses (MS2-phage and MNV-1).

The use of *E. coli* is less appropriate as indicator for Gram positive pathogens such as *L. monocytogenes*. In this case, the use of *Listeria* spp. could be more relevant. However, in the reviewed papers so far, mainly *E. coli* is applied as indicator to evaluate if the water treatment strategies are working properly, and no cross-contamination is occurring.

Bacteriophages could be used as an alternative for evaluating the potential effects of water treatments on enteric viruses. Presently, there are no recognised indicators for parasites. On the contrary, total bacterial counts (due to reaching high levels after initial contamination) are not reliable because they quickly reach high levels after the initial contamination and little changes during the production process are subsequently observed (Holvoet et al., 2012). Advantages and disadvantages of these and other microbial indicators such as spores of *C. perfringens* for parasites and specific phages for viruses can be found in the report from FAO and WHO (FAO and WHO, 2021a).

**Performance standard of process water**

As the operating procedure is targeted at maintaining the quality of the process water to avoid cross-contamination, the performance standard can be expressed as the avoidance of the accumulation of the target microbial indicator in the process water. As mentioned in Section 3.8.3, it was clarified that the microbiological quality of the water to avoid cross-contamination of the processed commodity needs to comply with the current limit for *E. coli* numbers (as indicator organisms). The reference of < 100 *E. coli* CFU/100 mL has been recommended. Luo et al. (2018) reported that, during commercial washing of chopped Romaine lettuce, shredded iceberg lettuce and diced cabbage, total bacterial survival showed a strong correlation with real-time FC concentration. 'Under approximately 10 mg/L, increasing FC significantly reduced the frequency and population of surviving bacteria detected. Increasing FC further resulted in the reduction of the aerobic plate count to below the detection limit (50 CFU/100 mL), except for a few sporadic positive samples with low cell counts. This study confirms that maintaining at least 10 mg/L FC in process water strongly reduced the likelihood of bacterial survival and thus potential cross-contamination of washed produce'. Their challenging study (Luo et al., 2011) using human pathogens at a pilot-scale level indicated that pathogen survival and cross-contamination frequently occurred when FC in solution dropped below 1 mg/L during the wash process. Similar findings were reported by Truchado et al. (2018) indicating that a *E. coli* level of 2.35 log CFU/100 mL was identified as a cut-off for the detection of pathogenic microorganisms in water with the smallest misclassification error in the classification tree analysis. This cut-off was able to correctly predict detection and non-detection of pathogens in water samples with 93% sensitivity and 66% specificity, respectively. The presence and absence predictive values were 74% and 90% respectively. Thus, for the water samples with levels of *E. coli* under 2.35 log CFU/100 mL there was a 90% probability that the water samples were not contaminated with pathogenic microorganism. On the other hand, almost three quarters of samples contaminated with *E. coli* at levels above 2.24 log CFU/100 mL were contaminated with pathogenic microorganisms. **In any case, the optimal situation is to set the critical limit for microbial indicators in a case-by-case basis, depending on the intended use of the process water.**

Water disinfection treatments aim to immediately inactivate pathogens introduced in the water via contaminated product before encountering the product. In this case, rather than the number of $\log_{10}$ reductions of the pathogen, the performance standard should also focus on the key parameters associated with the efficacy of the disinfectant used, on the residual amount of the disinfectant and, if relevant, specific physico-chemical parameters influencing this inactivation process.

In general, for chlorine-based treatments, a residual concentration of FC of 10 mg/L at a water pH < 5.5–6.5 is suggested as an approximate target, while for PAA based treatment 30–80 mg/L are reported (Gombas et al., 2017). However, these values are based on the available scientific studies usually performed at laboratory or pilot-plant scale. Therefore, other lower or higher process-specific targets may be required under large industrial scale depending on the product, equipment configuration and/or conditions of application of the disinfectant (López-Gálvez et al., 2021). This is why, the appropriate target microorganisms and the performance standards may be commodity and

process specific. Scientific justification of the identified performance standard must be provided e.g. based on internationally recognised guidance documents (Gombas et al., 2017; Section 3.7) and/or specific regulations/guidance documents (e.g. Section 3.1).

**[4] Design and execution of the validation study**

The design of a validation study must be tailored on a case-by-case basis and requires knowledge about the industrial handing and processing operations using process water, but also about the specificities of the processing facility and the process/es (step [2]). Moreover, an understanding of the factors determining the process performance and expertise on the methods and sensors for monitoring the relevant parameters and analysing the target microorganisms (considering the potential impact of sublethal damage of microorganisms, occurrence of VBNC cells, etc.) are needed for a proper interpretation of the results. On-site examination of the processing line/s and operations at each facility is highly recommended.

The parameters to be determined include fixed (unchanged) and variable parameters, both physico-chemical and microbiological, including those used to define the performance standards.

The sampling scheme including the location (spot in the process), the frequency of sampling and measurements should be adjusted to each parameter, the production volume and the duration of the processing operation to allow capturing the dynamics of the relevant parameters within a run/batch regarding the characteristics of the process water (temperature, pH), the accumulation of the organic matter (physico-chemical indicators described in Section 3.12) as well as the load of the microbial indicators.

Variability between batches can be covered through different independent trials (typically three performed in different days and if needed seasons) covering the range of conditions and parameters described in step [2]. The system should be cleaned and disinfected before carrying out the study and in between validation trials.

Sampling of process water may be complemented with the sampling of the product at the entrance (input) and exit (output) of the processing operation (e.g. incoming and washed produce) for microbiological testing, e.g. for enumeration of the microbial indicators. This result provides complementary information as the validation aim is to demonstrate the cross-contamination, not the inactivation (log reduction) of the contamination in the product.

Sampling methods, sample treatment (e.g. filtration) and analytical procedures should follow standardised protocols (i.e. ISO methods when available, validated tests). The validation study should also include the real-time measurements that will also be used for operational monitoring (temperature, pH, turbidity, etc. see Section 3.12 on monitoring) as well as off-line determinations that will be part of the verification procedures (microbiological testing and physico-chemical parameters, see Section 3.11.2 on verification). Calibrated and verified sensors and devises should be used (see Section 3.8).

In-plant validation studies cannot be performed through challenge testing with pathogens due to biosafety requirements. Working with pathogenic microorganisms must be done in dedicated laboratories and pilot-plants, which may not reproduce the specific industrial operating conditions to be validated. Challenge testing with non-pathogenic surrogates could be considered at industrial premises. However, suitable/qualified surrogates for validating the performance of ffFVHs handing and processing operations are presently lacking (Gombas et al., 2017). Therefore, specific studies will be needed to qualify a potential surrogate microorganism showing a similar behaviour as compared to the target pathogen (Deng et al., 2014). Moreover, challenge testing is costly, especially as all deviations of the process must be considered to cover the variability range of relevant operating conditions (as defined in step [2]) within and between production batches/runs. Therefore, in practice, validation of the process water management options will be carried out in the facilities using ffFVHs that are naturally contaminated with microbial indicators (Tudela et al., 2019a).

**[5] Reporting and setting handling and operating conditions**

All the conditions used, and the results obtained in each validation trial should systematically be recorded. The information of key parameters should show the actual boundaries of the operating conditions that were validated.

The results of the validation trials should provide the empiric evidence demonstrating the compliance with the performance standards of the relevant parameters (identified in step [3]) at the assessed range of operating conditions. Therefore, the results will allow to set the specific operating

conditions to be implemented in the rutinary commercial productions together with the key parameters and their critical limits.

When the validation study provides satisfactory results, the handing and processing operation is declared 'valid'. Once implemented for its routinely/commercial production, it must be monitored daily (see Section 3.12) and periodically verified (see the following Section 3.11.2).

When the conditions associated with the validated process(es) change in respect to the fixed parameters or regarding the boundaries of variable conditions used in the validation study, it should be assessed whether the system will be operating under the conditions originally validated and otherwise, a revalidation will need to be performed.

The outcome of the replies provided to the industry survey revealed that only 6 of 31 industries could fill in the questions related to their validation studies, two industries indicated 'not appropriate' because no water disinfection is applied and the remaining 23 FBOps did not fill in any information. From the six industries providing some information, a rather weak validation study of their water disinfection system can be derived: none of the industries is following an approach covering the five steps proposed above. Regarding the analytical determinations, physico-chemical parameters as temperature and pH are mentioned next to the concentration of free chlorine. Some industries combine the physico-chemical parameters with indicator organisms such as *Clostridia*, total viable count, coliforms or *E. coli*. Related to the frequency of validation, a scattered answer was given ranging from between twice a week, to monthly or every 6 months. Inclusion of the variability of the process was conducted by performing the validation in different seasons. In the design or set up of the validation experiments, two answers were recorded: based on technical advice and by sampling the commodity before and after the washing operation.

### 3.11.2. Relevant protocols for verification

Verification is conducted as part of a FSMS, to demonstrate that the applied water management strategies are being applied as required, and the process water reached the required microbiological quality (defined as fit-for-purpose for the intended use) to avoid cross-contamination of the ffFVHs via the water. Verification can be carried out by the FBOp and/or by the independent authority (e.g. external laboratory) and, together with reviewing/checking/auditing the monitoring records and the calibration status of measuring devices, typically includes also the microbiological testing of the process water.

The steps to set the verification of the microbiological quality of the process water used for handing and handling and processing operations of ffFVHs are outlined in Figure 12 and are described below.


**Figure 12:** Steps of the verification of the microbiological quality of the process water post-harvest handling and processing operations of ffFVHs

**[1] Determination of the microbiological and physico-chemical parameters for verification**

Verification approaches should tie to the validation carried out for each specific commodity and processing line. In this respect, as for validation, *E. coli* is considered the most suitable bacterial faecal indicator to assess the microbiological quality of the water in the verification procedures. Also, *Listeria* spp. as indicator for Gram positive pathogens can be applied. Bacteriophages (such as coliphages) are used as alternative to faecal indicator bacteria as indicators of human viral pathogens (Holvoet et al., 2012). Advantages and disadvantages of these and other microbial indicators can be found in the report from FAO and WHO (2021a).

In addition to the microbiological testing, determination of parameters associated with the load of organic matter may be complementary, particularly those related to the factors determining the performance of the water treatment that were included in the validation. Besides verifying the compliance of the physico-chemical parameters monitored in real-time (next section), physico-chemical parameters requiring off-line measurements (not suitable for monitoring purposes due to the time required to get the results) may be useful for verification purposes. For instance, the COD as indirect

measurement of organic content, indicates the level of organic and inorganic substances that can consume chlorine by measuring the oxygen required to oxidise soluble and particulate matter in water.

Total suspended solids, total phenolic content and total protein content are alternative parameters reported in the literature as chemical parameter indicators of the organic matter in the process water (Section 3.8.2).

**[2] Sampling sites and procedures**

The sampling regime should be feasible for the FBOp while at the same time providing the required information for the verification purposes. The critical sites and time within the operation for periodic verification testing should agree with those identified in the validation. Rotation of the sampling sites (at different times of the production day, in different days, in different seasons, in different production circumstances) contribute to cover a larger area and will give insight to a more robust verification procedure instead of always performing at the same day, same processing line, etc.

Standard procedures for water sampling (e.g. ISO methods) should be implemented. The volume of water to be sampled should be representative and in accordance with the requirements of the ISO standard.

**[3] Frequency of sampling for verification**

The frequency of sampling for microbiological and physico-chemical testing of process water should be established on a risk-based approach, with some flexibility.

The relatively high frequency of the initial verification procedures can be reduced and combined with rotation of sampling sites as satisfactory results are being obtained. Some recommendations:

- It should be clear that once a production process is well-established and historical data has been built up (see point e, trend observation, analysis) a lower frequency of verification activities can be established.
- An FBOp can, for instance, start with a relatively intense verification frequency and based upon the stability of the daily monitoring activities and the outcome of the verification activities, might decide to reduce the verification frequency on some production lines.
- Also, in case of a continuous production (e.g. long runs without shift to other commodities) the verification frequency can be decreased. However, in case of short runs, such as with multiple commodities on a process line or in case that no stable monitoring outcomes are achieved, the frequency of verification should be higher.
- For this risk-based approach, the outcome of the validation study will give also input (e.g. factors related to process, water treatment and commodities).

**[4] Analytical procedures**

Analysis of the water sampled for verification purposes may require concentration by membrane filtration. Standard membrane filtration procedures are described ISO methods for enumeration of microorganisms in water (ISO 19458:2006).[17] New techniques are being addressed in the scientific literature, particularly targeted to pathogens, which require higher sensitivity e.g. (Bissonnette et al., 2017; Bivins et al., 2020), though they are not recognised as official validated methods.

Microbial determinations can be done by culture and/or non-culture-based approaches. A review of the types of methods available for testing microbial quality of water is available in the FAO and WHO (2021a).

Culture based methods are considered the standard or reference methods (e.g. with ISO standards) for the enumeration of indicator bacteria by plate counting, most probable number (ISO 9308-2:2012)[18] and for the detection and identification of pathogens through enrichment-based procedures. To ensure the reliability of the results, they must be done in laboratory premises by technically qualified personnel following quality control procedures. Alternatives based on commercial test kits, ready-to-use for the FBOp are available for simple, rapid and cost-effective analysis of microbial indicators.

Non-culture based methods include a wide variety of molecular approaches based on the polymerase chain reaction (PCR), next generation sequencing (NGS) methods, immunological methods as well as optical biosensors, flow cytometry, etc. In general, they are considered more specific and

[17] ISO 19458:2006. Water quality – sampling for microbiological analysis. International Organization for Standardization. Geneva. Switzerland.

[18] ISO 9308-2:2012. Water quality – enumeration of *Escherichia coli* and coliform bacteria – Part 2: Most probable number method. International Organization for Standardization. Geneva. Switzerland.

can be faster compared to culture methods. Non-culture based methods are extensively used in the scientific studies, but they are not extensively implemented at FBOp level due to drawbacks related to limited official recognition (as standard validated method), the technical equipment and skills required.

Microorganisms in process water may be stressed by water treatments, suffering sublethal damage that inhibits their detection and quantification. VBNC are of special relevance, as they may result in an underestimation the actual concentration (see Sections 3.9.4 and 3.9.5).

Regarding the off-line physico-chemical parameters:

- the chemical oxygen demand (COD, mg $O_2$/L) is determined spectrophotometrically using a reactor digestion method based on the application of heat and a strong oxidising agent under acidic conditions to oxidise organic material to $CO_2$ and $H_2O$. The consumed oxidised agent is quantified.
- total suspended solids (mg) are determined by measuring dry weight of the material captured on a filter (after 2 h at 103°C).
- total phenolic content (by the Folin-Ciocaleu assay) or total protein content (by the spectrophotometric Bradford assay) are alternative parameters reported in the literature as chemical parameter indicators of the organic matter in the process water.

**[5] Trend analysis/trend observation**

Building up of historical information of the process robustness is an important activity for verification of the food safety management system of the FBOp. This means that all outcomes of a verification process, are used in trend observation (e.g. graphical representation per process line, per verification moment in a calendar year, etc.) or even a trend analysis (statistical analysis).

A mapping of the microbiological results of the indicator organism(s) combined with the additional physico-chemical parameters of the process water measured will allow for the FBOp to get insight into the robustness of the water management strategy applied.

In case it becomes clear that the verification outcomes are not satisfactory (i.e. not meeting the targets set in the validation, both for microbiological levels in the water and/or physico-chemical parameters), a review of the system is needed and, when necessary, a revalidation should be performed. So that the system can be amended or adopted to the circumstances and that new target parameters can be set.

The replies provided to the industry survey demonstrated inconsistencies regarding the verification activities of the applied water disinfection treatments. Only 12 of the 31 industries provide information about verification. Of these, some FBOps answered based on monitoring results (e.g. every hour a certain parameter is checked, but this is belonging to the operational monitoring and not to the industrial verification) and such answers were excluded. Two industries indicated 'not relevant' as an answer because no water disinfection treatment was applied. When considering the replies from the 12 industries providing some information, a combination of microbiological parameters in the water or product was applied (total count, coliforms, *E. coli*) in combination with physico-chemical parameters of the water (temperature, pH, concentration of biocide). Indicated frequency of verification activities ranged between monthly to once or twice a year. It can be concluded that based on the answers received verification of the applied water disinfection treatments is not being performed as required.

### 3.11.3. Main remarks

- In the context of process water for ffFVHs, the goal of the validation is obtaining evidence about the reliably achievable microbiological quality of the process water to avoid cross-contamination during the handling and processing operations. Validation procedures allow definition of the appropriate operational conditions associated with the water management strategy (e.g. performance standard of water disinfection and/or replenishment related to certain physico-chemical parameters of the process water) allowing to control the target microorganisms (e.g. generic *E. coli* as indicator organism).
- A validation procedure includes five steps:
    - defining the aim and scope of the validation;
    - defining operational conditions of process(es);
    - defining target microorganism/s and performance standards;
    - designing study design and execution of analysis;
    - establishing and monitoring SOPs.

- From the replies provided to the industry survey, a sound validation of the water disinfection treatments applied is currently lacking. Therefore, the actual performance of the applied water disinfection treatments can be questionable.
- Verification is conducted as part of a FSMS, to demonstrate that the applied water management strategies are being applied as required, and the process water ris achieving the microbiological quality (defined as fit-for-purpose for the intended use) to avoid cross-contamination of the ffFVHs via the water. Verification can be carried out by the FBOp and/or by the independent authority (e.g. external laboratory) and, together with reviewing/checking/ auditing the monitoring records and the calibration status of measuring devices, typically includes also the microbiological testing of the process water.
- For verification five steps are identified:
    - determination of the microbiological and physico-chemical parameters for verification,
    - sampling sites and procedure,
    - frequency of sampling for verification,
    - analytical procedures, and
    - trend observation/trend analysis.
- From the replies provided to the industry survey, a rather scattered situation regarding verification activities, both in set up, followed parameters, frequency etc. can be derived. Only 12 of 31 industries were indicating some information on their verification activities.
- The results of the EU survey illustrated the misunderstanding by the ffFVHs industry regarding the validation and verification.
- In literature scarce information is available on industrial validation and verification. Therefore, all information in this section is based upon scattered information from scientific papers, guidance documents and expert information.
- This leads to the situation that we cannot forecast e.g. necessary number of samples to be taken and sampling frequency for validation or verification, because these activities really need to be conducted at the level of each specific FBOp and processing line as a risk-based approach in the FBOp's FSMS (including their HACCP study).
- No direct pathogen detection in process water is recommended for validation nor for verification purposes, as their occurrence and levels are low. Indicator organisms (such as *E. coli* and *Listeria* spp.) for bacterial pathogens are well established. However *E. coli* and *Listeria* spp. are not suitable for parasites and viruses. Spores of *Clostridium perfringens* have been suggested as indicators for parasites and specific phages for viruses.

## 3.12. Relevant protocols (including parameters, analytical methods and frequency) to monitor the appropriate microbiological quality requirements of the water intended to be used for different post-harvest handling and processing operations of ffFVHs (AQ9)

The operational monitoring of the selected water management strategies is aiming at the follow-up of defined process parameters and conditions. Operational monitoring parameters should be selected from the evaluated factors in the validation study.

To have an efficacious operational monitoring, real-time information of the process parameters is necessary to be able to implement timely corrective actions when one of the parameters has breached the critical limits. The classical approach of using microbiological counts of the water as a monitoring method is not suitable, as it requires 24–48 h to obtain the results and hence does not give real-time information. Measurements can be done either off-line or on/in/at-line using calibrated devices.

Precision and accuracy of the methods should be assessed and recorded as part of the verification procedures described in the above Section 3.9, including periodic statistical evaluation of variances, the frequency of calibration of instrumentation, competency testing of the technicians, reagents used are within the method specification and shelf life (Gombas et al., 2017). The principle and the advantages and drawbacks of the different analytical devises/strategies were reviewed by (Albolafio et al., 2021). In Section 3.8, also the preventive measures to have a proper working of the applied water management system, including technical maintenance, calibration etc. was addressed.

The main relevant parameters useful for operational monitoring of process water belong to

(a) Process and product parameters.
(b) Water related parameters.
(c) Water disinfection treatment related parameters.

**Process and product parameters**

As the efficacy of the water management strategies are highly dependent on the operating conditions of the product being processed, during the routine production of batches the selected process parameters should be within the boundaries assessed in the validation study. These include process parameters that remain constant (fixed) in every processed batch as well as the conditions that may vary between batches and/or during a process (see Section 3.12.1, subheading [2]). There should be a manual or automatic system in place for each batch of ffFVHS being processed recording these data.

**Water related parameters**

Among the studied water related factors included in the validation, parameters selected in a case-by-case basis may include temperature and pH (Table 10) and load of organic matter (Table 11). In chlorine-based disinfection operations monitoring and controlling (adjusting) the temperature and the pH below 6.5 allow to maximise the presence of hypochlorous acid, which is the active component of the chlorine-based disinfectants (see Section 3.8). The temperature and the pH (López-Gálvez et al., 2019) are usually easily and quickly measurable with probes (off-line) or sensors (in/at line).

**Table 10:** Summary characteristics of relevant parameters for monitoring the water conditions

| Parameter | Nature | Means of real-time measurement | | Monitoring reason | Targets/critical limits |
|---|---|---|---|---|---|
| | | OFF-LINE (in some cases may not fulfil real-time requirements) | ON/IN/AT LINE | | |
| **Temperature (°C)** | Physical | Thermometer, data logger | Available commercial devices (sensor) | Factor determining the activity of oxidant-based disinfectants<br>Factor determining FC consumption by organic matter<br>Factor determining microbial growth | Refrigeration or ambient temperature (temperature limit depends on type of ffFVH) |
| **pH** | Physico-chemical | Probe (pH meter) or together with other parameters such as ORP, EC, temperature with multimeter | Available commercial devices (Sensor) | Factor determining the amount of undissociated (active) (chlorine)<br>Factor determining microbial growth | < 6.5 |

Monitoring of organic matter load can be done continuously by on/in-line systems providing real-time information on the status of the physiochemical parameters. Although off-line manual measurements can also provide quick results with sufficient frequency, there is a high possibility that the operation fell into failures in a timely manner to allow a rapid response. Regarding the monitoring of the accumulation of organic matter, numerous parameters are used (Section 3.9.2), some measurable by means of rapid and simple approaches suitable for operational monitoring (Table 12).

One parameter that can be linked for monitoring the load of organic matter the Total Organic Carbon (TOC), which measures the amount of carbon contained in the organic compounds. The main source of carbon in the process water originates from carbohydrates (Teng et al., 2018). Commercially available on-line monitoring systems of TOC are available, but they are costly and the applications are often limited by their range (e.g. below 50 mg/L) (Li et al., 2019).

The concentration of sugars present in the process water is defined as total soluble solids (TSS) and is measured as °Bri. To determine the TSS, off-line a portable refractometer can be used. However, more studies are needed to determine the correlation of this parameter with the estimation of the consumption of free chlorine mostly due to the low reactivity of sugars with chlorine (Toivonen and Lu, 2013).

Total Dissolved Solids (TDS) can be used as an indirect measurement of organic load by measuring the conductivity of the substances dissolved in the process water (Zhou et al., 2014; Luo et al., 2018). TDS have been used as an indicator for chlorine dosing, since the dissolved matter is the main contributor to chlorine consumption in drinking water (LeChevallier et al., 1981). However, considering that sugars are the main organic compounds in process water and they have a negligible conductivity, the capacity of TDS to predict changes in the organic load is limited (Teng et al., 2018). There are easy and inexpensive devices that can be used to determine TDS, such as TDSmeters.

Turbidity can be linked to the amount of particles present in the process water, as it measures the intensity of light scattered by the particles present in the water. Turbidity can be used as a rapid (seconds), easy and low cost measurement. However, the suitability of turbidity to predict the load of organic matter is not well defined since both organic and soil particles are capable of scattering light (LeChevallier et al., 1981).

Among all previously mentioned parameters, including oxidation reduction potential, protein content, phenolic content, pH, UVA at 254 nm (UVA254), COD and colour change, UVA254 showed promise for predicting chlorine demand in process water without biocides, as it was highly correlated with chlorine demand (Chen and Hung, 2016, 2017). The study of Van Haute et al. (2018) showed that UVA, with wavelengths between 240 and 290 nm, depending on the vegetable, can be used to predict chlorine demand. In addition, free chlorine itself influences UVA, and at a residual above 25 mg/L the chlorine interfered with the estimation of chlorine demand. UV/Vis at 320 nm was proposed for the routine water quality monitoring at on-farm root vegetable pack houses, being a sufficient measure for the water contamination with suspended, dissolved solids and organic matter monitoring. According to these materials' chemical limit values, the limit value of the absorption at A320 nm is 7.39–7.41 $cm^{-1}$ (Radzevičius et al., 2020). UV254 was identified as the most suitable wavelength for estimating organic load (Qi et al., 2020). The application of this parameter can have limitations due to the variability in the correlation between chlorine demand and UV absorbance among crops of the same vegetable and due to the possible interference by the pH regulators (López-Gálvez et al., 2021).

UV254 and turbidity are parameters that can be measured on-line in a washing tank and would be suitable indicators of the presence of organic matter in fresh produce wash water, as they correlate with COD.

**Table 11:** Summary characteristics of relevant parameters for monitoring the load organic matter

| Parameter | Nature | Means of real-time measurement |
|---|---|---|
| **Oxidation reduction potential (ORP, mV)** | Physico-chemical | Electrode (or together with pH) |
| **Total Organic Carbon (TOC)** | Physico-chemical | Test kits – colorimeter |
| **Total soluble solids (°Brix)** | Physical | Refractometer (portable devise) |
| **Total Dissolved Solids (TDS)** | Physical | TDSmeter (conductivity) |
| **Turbidity (NTU)** | Physical | Turbidimeter/Spectrophotometer ($A_{663}$) after filtering to remove suspended solids. Measures the light scattered by fine particles |
| **Absorbance UV254 ($cm^{-1}$)** | Physical | UV–Vis spectrophotometer after filtering wash water through a 0.45 μm pore-size filter |
| **Electrical Conductivity (EC, μS/cm)** | Physical | Measure of the concentration of ions present |
| **Maximum Filtrable Volume (MFV, mL)** | Physical | Quantification by sample pulling 50 mL through 0.45 μm membrane in 1 min at −80 kPa vacuum pump |

### Water disinfection treatment related parameters

Table 12 summarises the most relevant parameters and the analytical procedure allowing real-time measurement of residual disinfectants in the context of operational monitoring.

In case of chlorine-based disinfection treatments, the FC is the key parameter that needs to be monitored as it is the active form. Measurement of other chlorine related parameters (combined and/or total chlorine) may provide complementary information for a better understanding of the chlorine reaction dynamics.

**Table 12:** Summary characteristics of relevant parameters for monitoring the level of residual concentration of biocide based on chlorine (Gombas et al., 2017)

| Parameter | Nature | Means of real-time measurement |
|---|---|---|
| **Free chlorine residual disinfectant active form** | Chemical | Colorimetric titration methods<br>N,N-diethyl-p-phenylenediamine (DPD) methods<br>Manual colour wheels<br>Photometric instruments<br>Indirect electronic probesIon-specific electronic probes, including Amperometric chlorine sensors (on-line) |
| **Total chlorine residual disinfectant** | Chemical | Colour changing test strips |
| **PAA residual disinfectant** | Chemical | Colour changing test strips<br>Colorimetric titration methods (titration kit, drop titration kit, by permanganate, ceric sulfate and iodometric titration)<br>Photometric instruments<br>Amperometric sensors (on-line) |

The methods differ in terms of the chemical principle, cost, simplicity of application and technical characteristics (accuracy and precision) (Harp, 2003; Gombas et al., 2017). For free chlorine, test strips are the simplest method, however it shows the lower accuracy and precision and, in addition, measures the total chlorine and the pH of the water must be determined to ensure that the active chlorine species are predominant. DPD based methods are adopted by international standards. Due to the relative instability of chlorine, portable digital devices to be used on-site are preferable. In practice, the speed of the N,N-diethyl-p-phenylenediamine (DPD) photometric method using chlorine photometer devices makes it a suitable method for real time monitoring of free chlorine (Eaton and Franson, 2005; Van Haute et al., 2018).

For PAA, the most common test methods for rapid, cost effective and accurate routine measurement are based on the colorimetric titration (Gombas et al., 2017). Albolafio et al. (2021) compared different sensor-based methodologies for monitoring PAA residual concentration in process water. Using the iodometric based drop titration kit overestimated PAA because of the interferences, which requires time and trained personnel to ensure confidence in the results (Albolafio et al., 2021). A semi-permeable membrane amperometric probe placed in a flow cell was used as precise, *in-line* monitoring of PAA in samples of process water, which showed to underestimate the PAA concentration in lemon wash water because of the organic matter interference on the membrane (Albolafio et al., 2021). The chronoamperometric sensor and the online amperometric probe showed similar results (López-Gálvez et al., 2020). The reflectometric method using iodide ion as catalyst in the oxidation of chromogenic substrates to achieve selectivity for PAA in the presence of hydrogen peroxide are the basis of commercial rapid systems in form of disposable strips (Albolafio et al., 2021). Despite the fast measurement, an overestimation of the PAA concentration was obtained associated with a high content of suspended organic matter, which required the addition of high concentrations of PAA, coexisting with hydrogen peroxide (López-Gálvez et al., 2020; Albolafio et al., 2021).

The replies collected in the EU industry survey regarding the monitoring activities showed that the monitoring of the quality of the process water is absent or rather weak. Among the 4 out of 31 industries not applying any disinfection treatment to the process water, the quality of the water was not monitored. In one case they stated, 'According to the law and 2 times on *E. coli*". However, microbiological analysis cannot be a monitoring measurement due to the timelapse to obtain the results. Up to 27 industries apply a disinfection treatment to process water used for at least one of the processing operations. Among them, only four (15%) mentioned to have a continuous real-time monitoring put in place in at least one of the processing lines reported in the survey. In the four cases described, the disinfection is associated with the water used for the washing operation and, depending on the industry and the processing line (i.e. type of produce), the system continuously monitors the pH and ORP or the residual concentration of the biocide (i.e. free chlorine or peracetic acid) with regulated dosing to maintain the target residual concentration. Up to six (22%) respondents stated that they measure the residual biocide concentration every 2 h (five industries) or 3 h (1 industry), while three (11%) every 30 min (one industry) or 1 h (two industries) during the processing operation. Even though industry respondents considered these examples as real-time monitoring, some of the reported frequency of mesasurements may not allow the timely implementation of the corrective

actions in case of failure. However, the monitoring of the water quality was either absent or rather weak in most of the cases: six (22%) industries did not state any monitoring activity, while eight (30%) described the analysis of residual biocide (e.g. free chlorine, peracetic acid or peroxide) with a frequency ranging from every 8 h or twice a day, to daily or weekly. With these frequencies, such measurements cannot be considered monitoring as they do not allow a timely response for taking the corrective action before the (re)use of the process water. As already pointed out with validation and verification (Section 3.12), the survey illustrates the misunderstanding by the ffFVHs industry regarding the verification and monitoring activities as they are described in Table 9.

Moreover, despite 19 industries stated to apply corrective measures when the monitored parameters are out of the threshold, the responses were mostly restricted to 'yes', without describing the type of corrective measures.

### 3.12.1. Main remarks

- The operational monitoring of the selected water management strategies aims at the follow-up of defined process parameters and conditions. Operational monitoring parameters should be selected from the evaluated factors in the validation study.
- Relevant parameters useful for operational monitoring of process water belong to following categories:
    - Process and product parameters
    - Water related parameters
    - Water disinfection treatment related parameters
- To have an efficacious operational monitoring, a real-time information of the parameters is necessary to be able to have timely corrective actions when one of the parameters is out of its limits. The required real-time measurements can be done either off-line or with on/in/at-line methodologies using calibrated devices.
- Following up the microbiological counts of the water as operational monitoring method is not suitable, as it does not provide real-time information.
- In the literature scarce information is available on robustness of the real-time monitoring procedures under real industrial conditions and the performance of these procedures in case unexpected events occur.
- According to the industry survey, real-time monitoring of the quality of the process water using physico-chemical measurements is not widely implemented. None of the four industries not using a water disinfection treatment monitors the quality of the water. Among industries applying water disinfection treatments, continuous monitoring of the quality of process water, either through the pH and/or residual concentration of the biocide, was identified in few (4 out of 31) industries. However, the monitoring of the process water quality was either absent or rather weak in most of the cases, restricted to the measurements of the residual concentration of biocide (not any other physico-chemical parameter) at frequencies ranging from every 30 min or 1 h (three industries) to every 2 h or 3 h (six industries).
- The results of the EU survey illustrated the misunderstanding by the ffFVHs industry regarding the verification and monitoring activities, as sampling frequencies from every 8 h to weekly and/or e.g. microbiological analysis taking too long were wrongly considered as part of monitoring activities even if they do not allow a timely response for taking the corrective actions before the (re)use of the process water.

## 4. Conclusions

***TOR1 to describe the microbiological hazards associated with the use of water in post-harvest handling and processing operations of ffFVHs and the routes and rates of contamination of the water and the ffFVHs.***

**Answer to TOR 1.1:** Which are the most relevant microbiological hazards associated with the use of water in different post-harvest handling and processing operations for ffFVHs?

- The hazard prioritisation was based on EU reported outbreaks (2014–2020). These are often limited in the available data, e.g. without information on the step of the food supply chain where food product contamination occurred.

- The most relevant microbiological hazards identified are: (i) *L. monocytogenes, Salmonella* spp. and human pathogenic *E. coli,* which are found to contaminate a wide range of FVHs, impact morbidity (i.e. number of hospitalisations) and mortality (i.e. number of deaths), (ii) viruses, mostly norovirus, which have been identified in several viral outbreaks with frozen FVHs, especially frozen berries as implicated food vehicles; (iii) other hazards that have caused three or more European outbreaks associated with FVHs include *Yersinia* spp., *Shigella* spp. and *C. parvum*.

**TORs 1.2 and 1.3:** What are the routes of water contamination and the rates of contamination (increase in microbiological and pathogen load over time) for the most relevant microbiological hazards (identified in TOR 1.1.) in the water used in different post-harvest handling and processing operations for ffFVHs and between different ffFVHs batches?

**Answer to TOR 1.2 and TOR 1.3:**

- Most products can be contaminated in primary production, especially by zoonotic hazards transmitted faecal-orally, e.g. *Salmonella* spp., STEC, *C. parvum* and *Yersinia* spp. There was less information on the route of food-borne virus contamination, but enteric human viruses may contaminate the FVHs along the production chain. Hence, the FVHs are mostly contributing to water contamination by these hazards.
- In some listeriosis outbreaks, the outbreak investigations reported that contamination occurred at the processing plant; however, bacteria may also enter the processing plant from primary FVH in low (undetectable) numbers.
- Depending on the processing operation, all hazards can be accumulated in the process water and/or lead to batch-to-batch cross-contamination.
- Despite outbreak investigations, the route of contamination was seldom confirmed. Hazard occurrence data along the production chain did not provide evidence for a specific point of contamination for any hazard.
- The contamination rate of the process water is defined as a dynamic variable that describes the instantaneous change over a given time (usually increase), of microbial load in process water and ffFVHs entering the water and therefore, it requires a dynamic modelling approach leading to a high uncertainty.
- Factors affecting the contamination rate of process water include: (i) the number of microorganisms in the contaminated ffFVHs, (ii) the FVH:water ratio (w:v), (iii) the time period in which the same process water is used, as well as (iv) the transfer of microorganisms from product to water and, vice versa from the water to the product.
- Currently available mathematical models can be used to estimate the transfer dynamics of microorganisms, which needs to be done on a case-by-case basis, possibly grouping some ffFVHs and processing conditions that share similar contamination dynamics.

***TOR2 to describe specific intervention strategies (*i.e. *water disinfection treatments, water replenishment rates, good hygiene practices, etc.) needed to ensure the appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of ffFVHs, taking into account their impact on the physiological state of the microbiological hazards present in the water*.**

**Answer to ToR 2.1**: Which good hygiene practices are recommended to ensure appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of ffFVHs?

- Good hygienic practices are preventive measures taken by the FBOp as GHP/GMP as part of the PRP, to prevent contamination of the ffFVHs being processed. Identified Good Hygiene practices, related to a water management plan and implemented water management system, include:
    - Technical maintenance of infrastructure associated with water management systems,
    - Training staff in operational monitoring of water management strategies and
    - Cooling of post-harvest process water to reduce bacterial growth in the water.

**Answer to TOR 2.2:** Which are the most efficacious water disinfection treatments (dose and mode of application) to maintain the appropriate microbiological quality requirements of water used during different post-harvest handling and processing operations of ffFVHs?

- A water disinfection treatment is considered 'efficacious' when it can maintain the microbiological quality of the process water at a level that avoids microbiological cross-contamination of ffFVHs during handling and processing operations.
- The efficacy of water disinfection treatments depends on the specific processing operation conditions, including the initial water quality, the type of water disinfection treatment and the physico-chemical parameters of process water, i.e. concentration of organic and inorganic matter, pH and temperature. The extent to which each of these parameters affects the water disinfection treatment applied can vary.
- Chlorine-based disinfectants and PAA have been reported as common water disinfection treatments used to maintain the microbiological quality of process water of ffFVHs. Chlorine-based disinfectants have been shown to be efficacious under industrial conditions. No such evidence was found for PAA. The use of these, or any other water disinfection treatments, must be undertaken following an appropriate water management strategy (including validation, operational monitoring and verification to demonstrate their performance).
- The chlorine-based water management strategy (dose and mode of application) should be based on: (i) maintaining a sufficient level of residual FC instead of a target total chlorine level, (ii) identifying the minimum effective biocide concentration and (iii) avoiding the excessive use of the biocides.

**Answer to TOR 2.3:** What is the impact of different water disinfection treatments on the induction of the viable but non-cultivable (VBNC) state or injury state in bacteria in water used for different post-harvest handling and processing operations of ffFVHs?

- The different water disinfection treatments, including the concentration of biocides and contact times, can affect the induction of sub-lethally injured or VBNC cells of pathogenic bacteria and/ or their recovery. The impact thereof has not been comprehensively characterised, and quantitative data about these phenomena is considered a data gap.

**Answer to TOR 2.4:** Which are the relevant parameters to establish efficacious water replenishment rates needed to maintain the appropriate microbiological quality requirements of water used for different post-harvest handling and processing operations of ffFVHs?

- There is limited information available about water replenishment in post-harvest handling and processing operations of ffFVHs. Based on the available information no relevant parameters could be identified to establish efficacious water replenishment rates.

***TOR3 to describe relevant parameters to assess the appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of ffFVHs.***

**Answer to TOR 3.1:** Which relevant parameters can be used to validate and/or verify the appropriate microbiological quality requirements of the water intended to be used for different post-harvest handling and processing operations of ffFVHs?

- Validation aims to obtain evidence about the reliably achievable microbiological quality of the process water to avoid cross-contamination during the handling and processing operations.
- In the validation procedures, a combination of microbiological and physico-chemical parameters should be used. The relevant parameters (e.g. pH, ORP, residual concentration of disinfectant, *E. coli*, *Listeria* spp.) need to be selected on a case-by-case basis.
- Validation procedures define the appropriate operational conditions associated with the water management strategy (e.g. performance standard of water disinfection and/or replenishment related to certain physico-chemical parameters of the process water), allowing control of the target microorganisms.
- Verification aims to demonstrate that the applied water management strategies are working as intended and the process water has achieved the required microbiological quality (defined as fit-for-purpose for the intended use) to avoid cross-contamination of the ffFVHs via the water.
- In the verification procedures, a combination of indicator microorganisms and the evaluation of the physico-chemical parameters of the applied water management strategy should be used. The results of the physico-chemical parameters used for operational monitoring and the microbiological parameters will be reviewed (trend observation/analysis). No direct pathogen detection in process water is recommended for validation nor for verification purposes, as their

occurrence and levels is low. Indicator organisms (such as *E. coli* and *Listeria* spp.) for bacterial pathogens are well established. Spores of *C. perfringens* have been suggested as indicators for parasites and specific phages for viruses.

**Answer to TOR 3.2**: Which relevant parameters can be used to monitor the appropriate microbiological quality requirements of water that are being used during different post-harvest handling and processing operations for ffFVHs?

- The operational monitoring of the selected water management strategies aims at the follow-up of defined process parameters and conditions. Operational monitoring parameters should be selected on a case-by-case basis from the evaluated factors in the validation study.
- During operational monitoring, real-time information of process parameters is necessary to ensure timely corrective actions.
- Relevant parameters useful for operational monitoring of process water belong to the following categories:
    - Process and product parameters (e.g. type of commodity, amount of product being processed, mode of cut, etc)
    - Water related parameters (e.g. temperature, pH, COD, TOC)
    - Water disinfection treatment related parameters (e.g. residual biocide concentration in the active form)
- Current enumeration methods based on colony count for microbiological indicators or pathogens are not suitable for operational monitoring of the applied water management strategy.

## 5. Recommendation

- More data in outbreak investigation reports, such as the origin of the raw FVH and if it has been post-harvest processed as well as possible implications of different types of water as source for the implicated pathogen are needed.
- Most of the emerging microbiological hazards described in the literature are based on targeted methods (e.g. culture methods). More data based on untargeted methods (e.g. shotgun metagenomics) would be necessary for the identification of additional emerging microbiological hazards.
- Specific and clear guidelines should be made available for FBOps to clarify the requirements on how water disinfection treatments can be used in the context of maintaining the microbiological quality of water used in the post-harvest handling and processing operations of ffFVHs.
- Technical guidance is needed on the procedures for the validation, operational monitoring and verification of the intervention strategies that can be applied as part of the process water management system. Training of the FBOp and CA on the terminology, procedures and requirements of these validation, verification and operational monitoring procedures.
- The development and implementation of rapid microbiological tests for operational monitoring will provide a better control of process water quality.
- The generalised adoption of the risk based definition of 'fit-for-purpose water' would help FBOp to implement effective and sustainable water safety plans.

## References

Åberg R, Sjöman M, Hemminki K, Pirnes A, Räsänen S, Kalanti A, Pohjanvirta T, Caccio SM, Pihlajasaari A, Toikkanen S, Huusko S and Rimhanen-Finne R, 2015. *Cryptosporidium parvum* caused a large outbreak linked to frisée salad in Finland, 2012. Zoonoses Public Health, 62, 618–624. https://doi.org/10.1111/zph.12190

Abnavi MD, Kothapalli CR and Srinivasan P, 2021. Total amino acids concentration as a reliable predictor of free chlorine levels in dynamic fresh produce washing process. Food Chemistry, 335, 127651. https://doi.org/10.1016/j.foodchem.2020.127651

Afari GK, Liu H and Hung Y-C, 2019. The effect of produce washing using electrolyzed water on the induction of the viable but non-culturable (VBNC) state in *Listeria monocytogenes* and *Escherichia coli* O157:H7. LWT, 110, 275–282. https://doi.org/10.1016/j.lwt.2019.04.089

Aiyedun SO, Onarinde BA, Swainson M and Dixon RA, 2021. Foodborne outbreaks of microbial infection from fresh produce in Europe and North America: a systematic review of data from this millennium. International Journal of Food Science & Technology, 56, 2215–2223. https://doi.org/10.1111/ijfs.14884

Albolafio S, Tudela JA, Hernández N, Ortuño JA, Allende A and Gil MI, 2021. Practical applications of sensor-based methodologies for monitoring peracetic acid (PAA) as a disinfectant of fresh produce wash water. Food Control, 121, 107632. https://doi.org/10.1016/j.foodcont.2020.107632

Ali A, Yeoh WK, Forney C and Siddiqui MW, 2018. Advances in postharvest technologies to extend the storage life of minimally processed fruits and vegetables. Critical Reviews in Food Science and Nutrition, 58, 2632–2649. https://doi.org/10.1080/10408398.2017.1339180

Allende A and Gil M, 2014. Suitability of physical methods to assure produce safety. Stewart Postharvest Review, 10.

Allende A, Selma MV, Lopez-Galvez F and Gil MI, 2008a. Fresh-cut product sanitation and wash water disinfection: Problems and Solutions. Oral Communication. FOODMICRO 2008: Evolving microbial food quality and safety. Abstract. Publicación: Libro de resúmenes. Aberdeen, September 2008.

Allende A, Selma MV, López-Gálvez F, Villaescusa R and Gil MI, 2008b. Impact of wash water quality on sensory and microbial quality, including *Escherichia coli* cross-contamination, of fresh-cut escarole. Journal of Food Protection, 71, 2514–2518. https://doi.org/10.4315/0362-028x-71.12.2514

Arvaniti M, Tsakanikas P, Papadopoulou V, Giannakopoulou A and Skandamis P, 2021. *Listeria monocytogenes* sublethal injury and Viable-but-Nonculturable State induced by acidic conditions and disinfectants. Microbiology Spectrum, 9, e0137721. https://doi.org/10.1128/Spectrum.01377-21

Askari-Khorasgani O and Pessarakli M, 2020. Tomato (*Solanum lycopersicum*) culture in vermi-aquaponic systems: III. Strategies for sustainable and economic development: Co-cultivation with aquatic species. Journal of Plant Nutrition, 43, 1740–1756. https://doi.org/10.1080/01904167.2020.1739308

Avgoustaki DD and Xydis G, 2020. Chapter One - How energy innovation in indoor vertical farming can improve food security, sustainability, and food safety? In: MJ Cohen (ed). Advances in Food Security and Sustainability. Elsevier. pp. 1–51.

Ayrapetyan M, Williams TC, Baxter R and Oliver JD, 2015a. Viable but Nonculturable and persister cells coexist stochastically and are induced by human serum. Infection and Immunity, 83, 4194–4203. https://doi.org/10.1128/IAI.00404-15

Ayrapetyan M, Williams TC and Oliver JD, 2015b. Bridging the gap between viable but non-culturable and antibiotic persistent bacteria. Trends in Microbiology, 23, 7–13. https://doi.org/10.1016/j.tim.2014.09.004

Ayrapetyan M, Williams T and Oliver JD, 2018. Relationship between the Viable but Nonculturable State and antibiotic persister cells. Journal of Bacteriology, 200. https://doi.org/10.1128/jb.00249-18

Baloch MA, 2014. 24 - Leafy greens: the case study and real-life lessons from a Shiga-toxin-producing *Escherichia coli* (STEC) O145 outbreak in romaine lettuce. In: J Hoorfar (ed). Global Safety of Fresh Produce. Woodhead Publishing. pp. 340–355.

Banach JL, Sampers I, Van Haute S and van der Fels-Klerx HJ, 2015. Effect of disinfectants on preventing the cross-contamination of pathogens in fresh produce washing water. International Journal of Environmental Research and Public Health, 12, 8658–8677. https://doi.org/10.3390/ijerph120808658

Banach JL, Van Bokhorst-Van De Veen H, Van Overbeek LS, Van Der Zouwen PS, Van Der Fels-Klerx HJ and MNN G, 2017. The efficacy of chemical sanitizers on the reduction of *Salmonella* Typhimurium and *Escherichia coli* affected by bacterial cell history and water quality. Food Control, 81, 137–146. https://doi.org/10.1016/j.foodcont.2017.05.044

Banach JL, Van Bokhorst-Van De Veen H, Van Overbeek LS, Van Der Zouwen PS, Zwietering MH and van Der Fels-Klerx HJ, 2020. Effectiveness of a peracetic acid solution on *Escherichia coli* reduction during fresh-cut lettuce processing at the laboratory and industrial scales. International Journal of Food Microbiology, 321, 108537. https://doi.org/10.1016/j.ijfoodmicro.2020.108537

Banach JL, Zwietering MH and van der Fels-Klerx HJ, 2021. Multi-criteria decision analysis to evaluate control strategies for preventing cross-contamination during fresh-cut lettuce washing. Food Control, 128, 108136. https://doi.org/10.1016/j.foodcont.2021.108136

Bandi AC, Cristea V, Dediu L, Petrea SM, Cretu M, Turek Rahoveanu A, Zugravu AG, Rahoveanu MMT, Mocuta DN and Soare I, 2016. The review of existing and in-progress technologies of the different subsystems required for the structural and functional elements of the model of multi-purpose aquaponic production system. Romanian Biotechnology Letters, 21, 11621–11631.

Barrera MJ, Blenkinsop R and Warriner K, 2012. The effect of different processing parameters on the efficacy of commercial post-harvest washing of minimally processed spinach and shredded lettuce. Food Control, 25, 745–751. https://doi.org/10.1016/j.foodcont.2011.12.013

Barton Behravesh C, Mody RK, Jungk J, Gaul L, Redd JT, Chen S, Cosgrove S, Hedican E, Sweat D, Chávez-Hauser L, Snow SL, Hanson H, Nguyen TA, Sodha SV, Boore AL, Russo E, Mikoleit M, Theobald L, Gerner-Smidt P, Hoekstra RM, Angulo FJ, Swerdlow DL, Tauxe RV, Griffin PM and Williams IT, 2011. 2008 outbreak of Salmonella Saintpaul infections associated with raw produce. New England Journal of Medicine, 364, 918–927. https://doi.org/10.1056/NEJMoa1005741

Bayer C, Bernard H, Prager R, Rabsch W, Hiller P, Malorny B, Pfefferkorn B, Frank C, de Jong A, Friesema I, Stark K and Rosner B, 2014. An outbreak of *Salmonella* Newport associated with mung bean sprouts in Germany and The Netherlands, October to November 2011. Eurosurveillance, 19. https://doi.org/10.2807/1560-7917.es2014.19.1.20665

Behravesh CB, Blaney D, Medus C, Bidol SA, Phan Q, Soliva S, Daly ER, Smith K, Miller B, Taylor T Jr, Nguyen T, Perry C, Hill TA, Fogg N, Kleiza A, Moorhead D, Al-Khaldi S, Braden C and Lynch MF, 2012. Multistate outbreak of *Salmonella* serotype Typhimurium infections associated with consumption of restaurant tomatoes, USA, 2006: hypothesis generation through case exposures in multiple restaurant clusters. Epidemiology and Infection, 140, 2053–2061. https://doi.org/10.1017/s0950268811002895

Beuchat LR, 1996. Pathogenic microorganisms associated with fresh produce. Journal of Food Protection, 59, 204–216. https://doi.org/10.4315/0362-028x-59.2.204

Beuchat LR, Harris LJ, Ward TE and Kajs TM, 2001. Development of a proposed standard method for assessing the efficacy of fresh produce sanitizers. Journal of Food Protection, 64, 1103–1109. https://doi.org/10.4315/0362-028x-64.8.1103

Bilek SE and Turantaş F, 2013. Decontamination efficiency of high power ultrasound in the fruit and vegetable industry, a review. International Journal of Food Microbiology, 166, 155–162. https://doi.org/10.1016/j.ijfoodmicro.2013.06.028

Bissonnette L, Maheux AF and Bergeron MG, 2017. CRENAME, A molecular microbiology method enabling multiparametric assessment of potable/drinking water. Methods Molecular Biology, 1620, 141–151. https://doi.org/10.1007/978-1-4939-7060-5_9

Bivins A, Lowry S, Murphy HM, Borchardt M, Coyte R, Labhasetwar P and Brown J, 2020. Waterborne pathogen monitoring in Jaipur, India reveals potential microbial risks of urban groundwater supply. npj Clean. Water, 3, 35. https://doi.org/10.1038/s41545-020-00081-3

Blidariu F and Grozea A, 2011. Increasing the economical efficiency and sustainability of indoor fish farming by means of aquaponics - review. Scientific Papers: Animal Science and Biotechnologies, 44, 1–8.

Bozkurt H, Phan-Thien KY, van Ogtrop F, Bell T and McConchie R, 2020. Outbreaks, occurrence, and control of norovirus and hepatitis a virus contamination in berries: a review. Critical Reviews in Food Science and Nutrition, 61, 116–138. https://doi.org/10.1080/10408398.2020.1719383

Bracket RE, Ocasio W, Waters K, Barach J and Wan J, 2014. Validation and verification: a practical, industry-driven framework developed to support the requirements of the Food Safety Modernization Act (FSMA) of 2011. Food Protection Trends, 34, 410–425.

Buscaroli E, Braschi I, Cirillo C, Fargue-Lelievre A, Modarelli GC, Pennisi G, Righini I, Specht K and Orsini F, 2021. Reviewing chemical and biological risks in urban agriculture: a comprehensive framework for a food safety assessment of city region food systems. Food Control, 126, 108085. https://doi.org/10.1016/j.foodcont.2021.108085

Ceuppens S, Johannessen GS, Allende A, Tondo EC, El-Tahan F, Sampers I, Jacxsens L and Uyttendaele M, 2015. Risk factors for *Salmonella*, Shiga Toxin-Producing *Escherichia coli* and *Campylobacter* occurrence in primary production of leafy greens and strawberries. International Journal of Environmental Research and Public Health, 12, 9809–9831. https://doi.org/10.3390/ijerph120809809

Chen X and Hung Y-C, 2016. Predicting chlorine demand of fresh and fresh-cut produce based on produce wash water properties. Postharvest Biology and Technology, 120, 10–15. https://doi.org/10.1016/j.postharvbio.2016.05.007

Chen X and Hung Y-C, 2017. Effects of organic load, sanitizer pH and initial chlorine concentration of chlorine-based sanitizers on chlorine demand of fresh produce wash waters. Food Control, 77, 96–101. https://doi.org/10.1016/j.foodcont.2017.01.026

Chen Y, Burall LS, Luo Y, Timme R, Melka D, Muruvanda T, Payne J, Wang C, Kastanis G, Maounounen-Laasri A, De Jesus AJ, Curry PE, Stones R, K'Aluoch O, Liu E, Salter M, Hammack TS, Evans PS, Parish M, Allard MW, Datta A, Strain EA and Brown EW, 2016. Listeria monocytogenes in stone fruits linked to a multistate outbreak: enumeration of cells and whole-genome sequencing. Applied Environmental Microbiology, 82, 7030–7040. https://doi.org/10.1128/aem.01486-16

Codony F, Agustí G and Allué-Guardia A, 2015. Cell membrane integrity and distinguishing between metabolically active and inactive cells as a means of improving viability PCR. Molecular and Cellular Probes, 29, 190–192. https://doi.org/10.1016/j.mcp.2015.03.003

Colombe S, Jernberg C, Löf E, Angervall AL, Mellström-Dahlgren H, Dotevall L, Bengnér M, Hall I, Sundqvist L, Kühlmann-Berenzon S, Galanis I, Lindblad M, Hansen A and Rehn M, 2019. Outbreak of unusual H(2)S-negative monophasic *Salmonella* Typhimurium strain likely associated with small tomatoes, Sweden, August to October 2019. Eurosurveillance, 24. https://doi.org/10.2807/1560-7917.Es.2019.24.47.1900643

Colwell RR, 2009. Viable but Not Cultivable Bacteria. In: SS Epstein (ed). Uncultivated Microorganisms, Berlin, Heidelberg, Springer. pp. 121–129.

Cuevas-Ferrando E, Allende A, Pérez-Cataluña A, Truchado P, Hernández N, Gil MI and Sánchez G, 2021. Occurrence and accumulation of human enteric viruses and phages in process water from the fresh produce industry. Foods, 10, 1853. https://doi.org/10.3390/foods10081853

Da Silva Felicio MT, Hald T, Liebana E, Allende A, Hugas M, Nguyen-The C, Johannessen GS, Niskanen T, Uyttendaele M and McLauchlin J, 2015. Risk ranking of pathogens in ready-to-eat unprocessed foods of non-animal origin (FoNAO) in the EU: initial evaluation using outbreak data (2007–2011). International Journal of Food Microbiology, 195, 9–19. https://doi.org/10.1016/j.ijfoodmicro.2014.11.005

Dankwa AS, Machado RM and Perry JJ, 2020. Sources of food contamination in a closed hydroponic system. Letters in Applied Microbiology, 70, 55–62. https://doi.org/10.1111/lam.13243

Danyluk MD and Schaffner DW, 2011. Quantitative assessment of the microbial risk of leafy greens from farm to consumption: preliminary framework, data, and risk estimates. Journal of Food Protection, 74, 700–708. https://doi.org/10.4315/0362-028x.Jfp-10-373

Davidson GR, Kaminski CN and Ryser ET, 2014. Impact of organic load on *Escherichia coli* O157:H7 survival during pilot-scale processing of iceberg lettuce with acidified sodium hypochlorite. Journal of Food Protection, 77, 1669–1681. https://doi.org/10.4315/0362-028x.Jfp-14-067

De Bock T, Zhao X, Jacxsens L, Devlieghere F, Rajkovic A, Spanoghe P, Höfte M and Uyttendaele M, 2021. Evaluation of *B. thuringiensis*-based biopesticides in the primary production of fresh produce as a food safety hazard and risk. Food Control, 130, 108390. https://doi.org/10.1016/j.foodcont.2021.108390

De Corato U, 2020. Improving the shelf-life and quality of fresh and minimally-processed fruits and vegetables for a modern food industry: a comprehensive critical review from the traditional technologies into the most promising advancements. Critical Reviews in Food Science and Nutrition, 60, 940–975. https://doi.org/10.1080/10408398.2018.1553025

Deng K, Wang X, Yen LH, Ding H and Tortorello ML, 2014. Behavior of Shiga toxigenic *Escherichia coli* relevant to lettuce washing processes and consideration of factors for evaluating washing process surrogates. Journal of Food Protection, 77, 1860–1867. https://doi.org/10.4315/0362-028X.JFP-14-220

Dinu LD and Bach S, 2011. Induction of viable but nonculturable *Escherichia coli* O157:H7 in the phyllosphere of lettuce: a food safety risk factor. Applied and Environmental Microbiology, 77, 8295–8302. https://doi.org/10.1128/aem.05020-11

Dong K, Pan H, Yang D, Rao L, Zhao L, Wang Y and Liao X, 2020. Induction, detection, formation, and resuscitation of viable but non-culturable state microorganisms. Comprehensive Reviews in Food Science and Food Safety, 19, 149–183. https://doi.org/10.1111/1541-4337.12513

Dorn-In S, Gareis M and Schwaiger K, 2019. Differentiation of live and dead *Mycobacterium tuberculosis* complex in meat samples using PMA qPCR. Food Microbiology, 84, 103275. https://doi.org/10.1016/j.fm.2019.103275

Dreux N, Albagnac C, Federighi M, Carlin F, Morris CE and Nguyen-the C, 2007. Viable but non-culturable *Listeria monocytogenes* on parsley leaves and absence of recovery to a culturable state. Journal of Applied Microbiology, 103, 1272–1281. https://doi.org/10.1111/j.1365-2672.2007.03351.x

Du M, Xiao Z and Luo Y, 2022. Advances and emerging trends in cultivation substrates for growing sprouts and microgreens toward safe and sustainable agriculture. Current Opinion in Food Science, 46, 100863. https://doi.org/10.1016/j.cofs.2022.100863

Eaton AD and Franson MAH, 2005. Method 4500-Cl, G: DPD colorimetric method. Standard methods for the Examination of Water and Wastewater. 21st edn. American Public Health Association, Washington, DC.

ECDC and EFSA (European Centre for Disease Prevention and Control and European Food Safety Authority), 2013. Outbreak of Hepatitis A virus infection in four Nordic countries. 2397-8325. 417E pp https://doi.org/10.2903/sp.efsa.2013.EN-417

ECDC and EFSA (European Centre for Disease Prevention and Control and European Food Safety Authority), 2014. Outbreak of hepatitis A in EU/EEA countries – Second update. 2397-8325. 581E pp https://doi.org/10.2903/sp.efsa.2014.EN-581

ECDC and EFSA (European Centre for Disease Prevention and Control and European Food Safety Authority), 2021. Multi-country outbreak of Salmonella Braenderup ST22, presumed to be linked to imported melons - 20 July 2021. Stockholm: ECDC/EFSA; 2021 https://doi.org/10.2903/sp.efsa.2021.EN-6807

EFSA (European Food Safety Authority), 2014. Update of the technical specifications for harmonised reporting of food-borne outbreaks through the European Union reporting system in accordance with Directive 2003/99/EC. EFSA Journal 2014;12(3):3598, 25 pp. https://doi.org/10.2903/j.efsa.2014.3598

EFSA (European Food Safety Authority), Allende A, Barre L, Jacxsens L, Liebana E, Messens W, Sarno E and da Silva Felicio MT, 2018. Urgent scientific and technical assistance to provide recommendations for sampling and testing in the processing plants of frozen vegetables aiming at detecting *Listeria monocytogenes*. EFSA supporting publication 2018;15(7):EN-1445, 41 pp. https://doi.org/10.2903/sp.efsa.2018.EN-1445

EFSA (European Food Safety Authority), Martino L, Aiassa E, Halldórsson Þ, Koutsoumanis KP, Naegeli H, Baert K, Baldinelli F, Devos Y, Lodi F, Lostia A, Manini P, Merten C, Messens W, Rizzi V, Tarazona J, Titz A and Vos S, 2020. Draft framework for protocol development for EFSA's scientific assessments. EFSA supporting publication 2020;17(4):EN-1843, 46 pp. https://doi.org/10.2903/sp.efsa.2020.EN-1843

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2018a. Multi-country outbreak of *Listeria monocytogenes* serogroup IVb, multi-locus sequence type 6, infections linked to frozen corn and possibly to other frozen vegetables – first update. EFSA supporting publication 2018;EN-1448, 22 pp. https://doi.org/10.2903/sp.efsa.2018.EN-1448

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2018b. Multi-country outbreak of *Salmonella* Agona infections possibly linked to ready-to-eat food. EFSA supporting publication 2018;15(7):EN-1465, 17 pp. https://doi.org/10.2903/sp.efsa.2018.EN-1465

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2011. Scientific Opinion on the risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria in seeds and sprouted seeds. EFSA Journal 2011;9(11):2424, 101 pp. https://doi.org/10.2903/j.efsa.2011.2424

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013. Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 1 (outbreak data analysis and risk ranking of food/pathogen combinations). EFSA Journal 2013;11(1):3025, 138 pp. https://doi.org/10.2903/j.efsa.2013.3025

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014a. Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella* and Norovirus in berries). EFSA Journal 2014;12(6):3706, 95 pp. https://doi.org/10.2903/j.efsa.2014.3706

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014b. Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella* and Norovirus in leafy greens eaten raw as salads). EFSA Journal 2014;12(3):3600, 118 pp. https://doi.org/10.2903/j.efsa.2014.3600

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014c. Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella* and Norovirus in tomatoes). EFSA Journal 2014;12(10):3832, 75 pp. https://doi.org/10.2903/j.efsa.2014.3832

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014d. Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella* in melons). EFSA Journal 2014;12(10):3831, 77 pp. https://doi.org/10.2903/j.efsa.2014.3831

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014e. Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella*, *Yersinia*, *Shigella* and Norovirus in bulb and stem vegetables, and carrots). EFSA Journal 2014;12(12):3937, 91 pp. https://doi.org/10.2903/j.efsa.2014.3937

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2016. Scientific opinion on the risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs. EFSA Journal 2016;14( 7):4524, 93 pp. https://doi.org/10.2903/j.efsa.2016.4524

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2020. The public health risk posed by *Listeria monocytogenes* in frozen fruit and vegetables including herbs, blanched during processing. EFSA Journal 2020;18(4):6092, 102 pp. https://doi.org/10.2903/j.efsa.2020.6092

Einöder-Moreno M, Lange H, Grepp M, Osborg E, Vainio K and Vold L, 2016. Non-heat-treated frozen raspberries the most likely vehicle of a norovirus outbreak in Oslo, Norway, November 2013. Epidemiology and Infection, 144, 2765–2772. https://doi.org/10.1017/s0950268816000194

Espenhain L, Riess M, Müller L, Colombe S, Ethelberg S, Litrup E, Jernberg C, Kühlmann-Berenzon S, Lindblad M, Hove NK, Torpdahl M and Mörk MJ, 2019. Cross-border outbreak of *Yersinia enterocolitica* O3 associated with imported fresh spinach, Sweden and Denmark, March 2019. Eurosurveillance, 24, 1900368. https://doi.org/10.2807/1560-7917.ES.2019.24.24.1900368

Fallon SD, Rios DV and Fonseca JM, 2011. Scrutinizing the sanitizing process involving aqueous chlorine from field to fresh-cut iceberg lettuce. Acta Horticulturae, 906, 37–42.

Fan X and Sokorai KJ, 2015. Formation of trichloromethane in chlorinated water and fresh-cut produce and as a result of reaction with citric acid. Postharvest Biology and Technology, 109, 65–72. https://doi.org/10.1016/j.postharvbio.2015.06.009

FAO and RUAF, 2022. Urban and peri-urban agriculture sourcebook - from production to food systems. FAO and Rikolto, Rome. 156 pp. https://doi.org/10.4060/cb9722en

FAO and WHO (Food and Agriculture Organization of the United Nations and World Health Organization), 2008. Microbiological Hazards in Fresh Leafy Vegetables and Herbs: Meeting Report. Microbiological Risk Assessment Series No. 14. Rome. 154 pp Available online: https://www.fao.org/3/i0452e/i0452e.pdf

FAO and WHO (Food and Agriculture Organization of the United Nations and World Health Organization), 2019. Safety and quality of water used in food production and processing – meeting report. Microbiological Risk Assessment Series 33. Rome. 100 pp Available online: https://www.fao.org/documents/card/fr/c/ca6062en/

FAO and WHO (Food and Agriculture Organization of the United Nations and World Health Organization), 2021a. Safety and quality of water used with fresh fruits and vegetables. Microbiological Risk Assessment Series No. 37. Rome. 153 pp https://doi.org/10.4060/cb7678en

FAO and WHO (Food and Agriculture Organization of the United Nations and World Health Organization), 2021b. Summary report of the Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment on the Prevention and Control of Microbiological Hazards in Fresh Fruits and Vegetables (Part 1: Administrative procedures, meeting scope/objectives, data collection; Part 2 General principle and fresh fruits and vegetables). 11 pp. Available online: https://www.fao.org/3/cb7664en/cb7664en.pdf.

FAO and WHO (Food and Agriculture Organization of the United Nations and World Health Organization), 2023. Prevention and control of microbiological hazards in fresh fruits and vegetables – Part 3: Sprout. Meeting report. Microbiological Risk Assessment Series No. 43. Rome, FAO https://doi.org/10.4060/cc3810en

Fields A, Wellman A, Lambert H, Viazis S, Kuntz T, Cloyd T, Parish M, Gieraltowski L, Heiman K and Harvey RR, 2015. P1-116 A Multistate outbreak of multiple *Salmonella* serotypes linked to organic sprouted chia powder products. Abstracts IAFP 2015 Portland, July 25-28, Oregon. Journal of Food Protection, 78, 108.

Fleischmann S, Robben C, Alter T, Rossmanith P and Mester P, 2021. How to evaluate non-growing cells — current strategies for determining antimicrobial resistance of VBNC bacteria. Antibiotics, 10.

Fu T-J, Li Y, Awad D, Zhou T-Y and Liu L, 2018. Factors affecting the performance and monitoring of a chlorine wash in preventing *Escherichia coli* O157:H7 cross-contamination during postharvest washing of cut lettuce. Food Control, 94, 212–221. https://doi.org/10.1016/j.foodcont.2018.06.035

Fusco V, Abriouel H, Benomar N, Kabisch J, Chieffi D, Cho G-S and Franz CMAP, 2018. Chapter 10 - Opportunistic Food-Borne Pathogens. In: AM Grumezescu and AM Holban (eds). Food Safety and Preservation. Academic Press. pp. 269–306.

Gajraj R, Pooransingh S, Hawker JI and Olowokure B, 2012. Multiple outbreaks of *Salmonella* Braenderup associated with consumption of iceberg lettuce. International Journal of Environmental Health Research, 22, 150–155. https://doi.org/10.1080/09603123.2011.613114

Gensberger ET, Polt M, Konrad-Köszler M, Kinner P, Sessitsch A and Kostić T, 2014. Evaluation of quantitative PCR combined with PMA treatment for molecular assessment of microbial water quality. Water Research, 67, 367–376. https://doi.org/10.1016/j.watres.2014.09.022

Gil MI, 2021. Management of preharvest and postharvest factors related to quality and safety aspects of leafy vegetables. Proceedings of the, 1–12 pp. https://doi.org/10.17660/ActaHortic.2021.1319.1

Gil MI, Selma MV, López-Gálvez F and Allende A, 2009. Fresh-cut product sanitation and wash water disinfection: problems and solutions. International Journal of Food Microbiology, 134, 37–45. https://doi.org/10.1016/j.ijfoodmicro.2009.05.021

Gilbert PA and Pettigrew R, 1984. Surfactants and the environment. International Journal of Cosmetic Science, 6, 149–158. https://doi.org/10.1111/j.1467-2494.1984.tb00371.x

Gombas D, Luo Y, Brennan J, Shergill G, Petran R, Walsh R, Hau H, Khurana K, Zomorodi B, Rosen J, Varley R and Deng K, 2017. Guidelines to validate control of cross-contamination during washing of fresh-cut leafy vegetables. Journal of Food Protection, 80, 312–330. https://doi.org/10.4315/0362-028x.Jfp-16-258

Gomes Neto NJ, Lucena Pessoa RM, Barbosa Nunes Queiroga IM, Magnani M, de Sousa Freitas FI, de Souza EL and Maciel JF, 2012. Bacterial counts and the occurrence of parasites in lettuce (*Lactuca sativa*) from different cropping systems in Brazil. Food Control, 28, 47–51. https://doi.org/10.1016/j.foodcont.2012.04.033

Gómez-López VM, Lannoo A-S, Gil MI and Allende A, 2014. Minimum free chlorine residual level required for the inactivation of *Escherichia coli* O157:H7 and trihalomethane generation during dynamic washing of fresh-cut spinach. Food Control, 42, 132–138. https://doi.org/10.1016/j.foodcont.2014.01.034

Gu G, Bolten S, Mowery J, Luo Y, Gulbronson C and Nou X, 2020. Susceptibility of food-borne pathogens to sanitizers in produce rinse water and potential induction of viable but non-culturable state. Food Control, 112, 107138. https://doi.org/10.1016/j.foodcont.2020.107138

Guzman-Herrador B, Vold L, Comelli H, MacDonald E, Heier BT, Wester AL, Stavnes TL, Jensvoll L, Lindegard Aanstad A, Severinsen G, Aasgaard Grini J, Werner Johansen Ø, Cudjoe K and Nygard K, 2011. Outbreak of *Shigella sonnei* infection in Norway linked to consumption of fresh basil, October 2011. Eurosurveillance, 16.

Harfield S, Beazley R, Denehy E, Centofanti A, Dowsett P, Housen T and Flood L, 2019. An outbreak and case-control study of *Salmonella* Havana linked to alfalfa sprouts in South Australia, 2018. Communicable Diseases Intelligence, 2018, 43. https://doi.org/10.33321/cdi.2019.43.45

Harp DL, 2003. Current Technology of Chlorine Analysis for Water and Wastewater. Technical Infomation Series - Booklet No. 17 Hach Company. Available online: https://docplayer.net/4276660-Current-technology-of-chlorine-analysis-for-water-and-wastewater.html

Hasan JA, Huq A, Nair GB, Garg S, Mukhopadhyay AK, Loomis L, Bernstein D and Colwell RR, 1995. Development and testing of monoclonal antibody-based rapid immunodiagnostic test kits for direct detection of *Vibrio cholerae* O139 synonym Bengal. Journal of Clinical Microbiology, 33, 2935–2939. https://doi.org/10.1128/jcm.33.11.2935-2939.1995

Hassan R, Rounds J, Sorenson A, Leos G, Concepción-Acevedo J, Griswold T, Tesfai A, Blessington T, Hardy C and Basler C, 2017. Multistate Outbreak of *Salmonella* Anatum Infections Linked to Imported Hot Peppers — United States, May–July 2016. MMWR. Morbidity and Mortality Weekly Report, 66, 663–667. https://doi.org/10.15585/mmwr.mm6625a2

Highmore CJ, Warner JC, Rothwell SD, Wilks SA and Keevil CW, 2018. Viable-but-Nonculturable *Listeria monocytogenes* and *Salmonella enterica* serovar Thompson induced by chlorine stress remain infectious. mBio, 9. https://doi.org/10.1128/mbio.00540-18

Holvoet K, Jacxsens L, Sampers I and Uyttendaele M, 2012. Insight into the prevalence and distribution of microbial contamination to evaluate water management in the fresh produce processing industry. Journal of Food Protection, 75, 671–681. https://doi.org/10.4315/0362-028x.Jfp-11-175

ICMSF (International Commission on Microbiological Specifications for Foods), 2011a. Fruits and fruit products. In: KMJ Swanson (ed). Microorganisms in foods 8 - Use of data for assessing process control and product acceptance. Springer, Boston, MA. pp. 177–195. https://doi.org/10.1007/978-1-4419-9374-8_13

ICMSF (International Commission on Microbiological Specifications for Foods), 2011b. Validation of control measures. In: KMJ Swanson (ed). Microorganisms in foods 8 - Use of data for assessing process control and product acceptance. Springer. pp. 13–32.

ICMSF (International Commission on Microbiological Specifications for Foods), 2011c. Vegetables and vegetable products. In: Swanson KMJ (ed.). Microorganisms in foods 8 - Use of data for assessing process control and product acceptance. Springer, Boston, MA. pp. 147–176. https://doi.org/10.1007/978-1-4419-9374-8_12

ICMSF (International Commission on Microbiological Specifications for Foods), 2011d. Verification of environmental control measures. In: KMJ Swanson (ed). Microorganisms in foods 8 - Use of data for assessing process control and product acceptance. Springer. pp. 41–46.
ICMSF (International Commission on Microbiological Specifications for Foods), 2011e. Verification of process control. In: KMJ Swanson (ed). Microorganisms in foods 8 - Use of data for assessing process control and product acceptance. Springer. pp. 33–40.
ICMSF (International Commission on Microbiological Specifications for Foods), 2018. Enterohemorrhagic *Escherichia coli* on fresh-cut leafy vegetables. Microorganisms in foods 7: microbiological testing in food safety management. Springer International Publishing. pp. 385–410. 978-3-319-68460-4. https://doi.org/10.1007/978-3-319-68460-4_17
Jacxsens L, Luning PA, van der Vorst JGAJ, Devlieghere F, Leemans R and Uyttendaele M, 2010. Simulation modelling and risk assessment as tools to identify the impact of climate change on microbiological food safety – the case study of fresh produce supply chain. Food Research International, 43, 1925–1935. https://doi.org/10.1016/j.foodres.2009.07.009
Kaletta T and Hengartner MO, 2006. Finding function in novel targets: *C. elegans* as a model organism. Nature Review Drug Discovery, 5, 387–398. https://doi.org/10.1038/nrd2031
Kell DB, Kaprelyants AS, Weichart DH, Harwood CR and Barer MR, 1998. Viability and activity in readily culturable bacteria: a review and discussion of the practical issues. Antonie Van Leeuwenhoek, 73, 169–187. https://doi.org/10.1023/a:1000664013047
Keuckelaere A, Jacxsens L, Amoah P, Medema G, McClure P, Jaykus L-A and Uyttendaele M, 2015. Zero risk does not exist: lessons learned from microbial risk assessment related to use of water and safety of fresh produce. Comprehensive Reviews in Food Science and Food Safety, 14, 387–410. https://doi.org/10.1111/1541-4337.12140
Kozai T, 2016. Plant production process, floor plan, and layout of PFAL. pp. 203–212.
Kwan PS, Xavier C, Santovenia M, Pruckler J, Stroika S, Joyce K, Gardner T, Fields PI, McLaughlin J, Tauxe RV and Fitzgerald C, 2014. Multilocus sequence typing confirms wild birds as the source of a *Campylobacter* outbreak associated with the consumption of raw peas. Applied and Environmental Microbiology, 80, 4540–4546. https://doi.org/10.1128/aem.00537-14
Kyriacou MC, Rouphael Y, Di Gioia F, Kyratzis A, Serio F, Renna M, De Pascale S and Santamaria P, 2016. Micro-scale vegetable production and the rise of microgreens. Trends in Food Science & Technology, 57, 103–115. https://doi.org/10.1016/j.tifs.2016.09.005
Launders N, Locking ME, Hanson M, Willshaw G, Charlett A, Salmon R, Cowden J, Harker KS and Adak GK, 2016. A large Great Britain-wide outbreak of STEC O157 phage type 8 linked to handling of raw leeks and potatoes. Epidemiology and Infection, 144, 171–181. https://doi.org/10.1017/s0950268815001016
LeChevallier MW, Evans TM and Seidler RJ, 1981. Effect of turbidity on chlorination efficiency and bacterial persistence in drinking water. Applied and Environmental Microbiology, 42, 159–167.
Lee S and Lee J, 2015. Beneficial bacteria and fungi in hydroponic systems: types and characteristics of hydroponic food production methods. Scientia Horticulturae, 195, 206–215. https://doi.org/10.1016/j.scienta.2015.09.011
Léonard L, Bouarab Chibane L, Ouled Bouhedda B, Degraeve P and Oulahal N, 2016. Recent advances on multi-parameter flow cytometry to characterize antimicrobial treatments. Frontiers in Microbiology, 7, 1225. https://doi.org/10.3389/fmicb.2016.01225
Li L, Mendis N, Trigui H, Oliver JD and Faucher SP, 2014. The importance of the viable but non-culturable state in human bacterial pathogens. Frontiers in Microbiology, 5. https://doi.org/10.3389/fmicb.2014.00258
Li J, Teng Z, Weng S, Zhou B, Turner ER, Vinyard BT and Luo Y, 2019. Dynamic changes in the physicochemical properties of fresh-cut produce wash water as impacted by commodity type and processing conditions. PLOS ONE, 14, e0222174. https://doi.org/10.1371/journal.pone.0222174
Lienemann T, Niskanen T, Guedes S, Siitonen A, Kuusi M and Rimhanen-Finne R, 2011. Iceberg lettuce as suggested source of a nationwide outbreak caused by two *Salmonella* serotypes, Newport and Reading, in Finland in 2008. Journal of Food Protection, 74, 1035–1040. https://doi.org/10.4315/0362-028x.Jfp-10-455
López-Gálvez F, Allende A, Selma MV and Gil MI, 2009. Prevention of Escherichia coli cross-contamination by different commercial sanitizers during washing of fresh-cut lettuce. International Journal of Food Microbiology, 133, 167–171. https://doi.org/10.1016/j.ijfoodmicro.2009.05.017
Lopez-Galvez F, Allende A, Pedrero-Salcedo F, Alarcon JJ and Gil MI, 2014. Safety assessment of greenhouse hydroponic tomatoes irrigated with reclaimed and surface water. International Journal of Food Microbiology, 191, 97–102. https://doi.org/10.1016/j.ijfoodmicro.2014.09.004
López-Gálvez F, Tudela JA, Allende A and Gil MI, 2019. Microbial and chemical characterization of commercial washing lines of fresh produce highlights the need for process water control. Innovative Food Science and Emerging Technologies, 51, 211–219. https://doi.org/10.1016/j.ifset.2018.05.002
López-Gálvez F, Truchado P, Tudela JA, Gil MI and Allende A, 2020. Critical points affecting the microbiological safety of bell peppers washed with peroxyacetic acid in a commercial packinghouse. Food Microbiology, 88, 103409. https://doi.org/10.1016/j.fm.2019.103409

López-Gálvez F, Allende A and Gil MI, 2021. Recent progress on the management of the industrial washing of fresh produce with a focus on microbiological risks. Current Opinion in Food Science, 38, 46–51. https://doi.org/10.1016/j.cofs.2020.10.026

López-Velasco G, Tomás-Callejas A, Sbodio A, Artés-Hernández F and Suslow TV, 2012. Chlorine dioxide dose, water quality and temperature affect the oxidative status of tomato processing water and its ability to inactivate *Salmonella*. Food Control, 26, 28–35. https://doi.org/10.1016/j.foodcont.2011.12.016

Luna S, Taylor M, Galanis E, Asplin R, Huffman J, Wagner D, Hoang L, Paccagnella A, Shelton S, Ladd-Wilson S, Seelman S, Whitney B, Elliot E, Atkinson R, Marshall K and Basler C, 2018. Outbreak of *Salmonella* Chailey infections linked to precut coconut pieces - United States and Canada, 2017. MMWR Morbidity and Mortality Weekly Report, 67, 1098–1100. https://doi.org/10.15585/mmwr.mm6739a5

Luo Y, 2007. Fresh-cut produce wash water reuse affects water quality and packaged product quality and microbial growth in romaine lettuce. HortScience horts, 42, 1413–1419. https://doi.org/10.21273/HORTSCI.42.6.1413

Luo Y, Nou X, Yang Y, Alegre I, Turner E, Feng H, Abadias M and Conway W, 2011. Determination of free chlorine concentrations needed to prevent *Escherichia coli* O157:H7 cross-contamination during fresh-cut produce wash. Journal of Food Protection, 74, 352–358. https://doi.org/10.4315/0362-028x.Jfp-10-429

Luo Y, Nou X, Millner P, Zhou B, Shen C, Yang Y, Wu Y, Wang Q, Feng H and Shelton D, 2012. A pilot plant scale evaluation of a new process aid for enhancing chlorine efficacy against pathogen survival and cross-contamination during produce wash. International Journal of Food Microbiology, 158, 133–139. https://doi.org/10.1016/j.ijfoodmicro.2012.07.008

Luo Y, Zhou B, Van Haute S, Nou X, Zhang B, Teng Z, Turner ER, Wang Q and Millner PD, 2018. Association between bacterial survival and free chlorine concentration during commercial fresh-cut produce wash operation. Food Microbiology, 70, 120–128. https://doi.org/10.1016/j.fm.2017.09.013

Lv R, Wang K, Feng J, Heeney DD, Liu D and Lu X, 2020. Detection and quantification of Viable but Non-culturable *Campylobacter jejuni*. Frontiers in Microbiology, 10, 2920. https://doi.org/10.3389/fmicb.2019.02920

MacDonald E, Einöder-Moreno M, Borgen K, Thorstensen Brandal L, Diab L, Fossli Ø, Guzman Herrador B, Hassan AA, Johannessen GS, Johansen EJ, Jørgensen Kimo R, Lier T, Paulsen BL, Popescu R, Tokle Schytte C, Sæbø Pettersen K, Vold L, Ørmen Ø, Wester AL, Wiklund M and Nygård K, 2016. National outbreak of *Yersinia enterocolitica* infections in military and civilian populations associated with consumption of mixed salad, Norway, 2014. Eurosurveillance, 21. https://doi.org/10.2807/1560-7917.Es.2016.21.34.30321

Machado-Moreira B, Richards K, Brennan F, Abram F and Burgess CM, 2019. Microbial contamination of fresh produce: what, where, and how? Comprehensive Reviews in Food Science and Food Safety, 18, 1727–1750. https://doi.org/10.1111/1541-4337.12487

Mäde D, Trübner K, Neubert E, Höhne M and Johne R, 2013. Detection and typing of Norovirus from frozen strawberries involved in a large-scale gastroenteritis outbreak in Germany. Food and Environmental Virology, 5, 162–168. https://doi.org/10.1007/s12560-013-9118-0

Manzocco L, Ignat A, Bartolomeoli I, Maifreni M and Nicoli MC, 2015. Water saving in fresh-cut salad washing by pulsed light. Innovative Food Science & Emerging Technologies, 28, 47–51. https://doi.org/10.1016/j.ifset.2015.01.006

Marín A, Tudela JA, Garrido Y, Albolafio S, Hernández N, Andújar S, Allende A and Gil MI, 2020. Chlorinated wash water and pH regulators affect chlorine gas emission and disinfection by-products. Innovative Food Science & Emerging Technologies, 66, 102533. https://doi.org/10.1016/j.ifset.2020.102533

Martín-Belloso O, Soliva-Fortuny R and Oms-Oliu G, 2012. Fresh-Cut Fruits. In: NK Sinha, JS Sidhu, J Barta, JSB Wu and MB Cano (eds). Handbook of Fruits and Fruit Processing. 2nd edn. John Wiley & Sons, Ltd. pp. 245–262.

Marus JR, Bidol S, Altman SM, Oni O, Parker-Strobe N, Otto M, Pereira E, Buchholz A, Huffman J, Conrad AR and Wise ME, 2019. Notes from the field: outbreak of listeriosis likely associated with prepackaged caramel apples - United States, 2017. MMWR Morbidity and Mortality Weekly Report, 68, 76–77. https://doi.org/10.15585/mmwr.mm6803a5

McCollum JT, Cronquist AB, Silk BJ, Jackson KA, O'Connor KA, Cosgrove S, Gossack JP, Parachini SS, Jain NS, Ettestad P, Ibraheem M, Cantu V, Joshi M, DuVernoy T, Fogg NW Jr, Gorny JR, Mogen KM, Spires C, Teitell P, Joseph LA, Tarr CL, Imanishi M, Neil KP, Tauxe RV and Mahon BE, 2013. Multistate outbreak of listeriosis associated with cantaloupe. New England Journal of Medicine, 369, 944–953. https://doi.org/10.1056/NEJMoa1215837

McEntire RC, McEntire C, Lovelace T and Morris D, 2016. inventors. McEntire Produce, Inc., assignee. 2016. System and process for processing fresh produce. US Patent No. 9, 326,543 B2.

McKerr C, Adak GK, Nichols G, Gorton R, Chalmers RM, Kafatos G, Cosford P, Charlett A, Reacher M, Pollock KG, Alexander CL and Morton S, 2015. An outbreak of *Cryptosporidium parvum* across England and Scotland associated with consumption of fresh pre-cut salad leaves, May 2012. PLOS ONE, 10, e0125955. https://doi.org/10.1371/journal.pone.0125955

Meireles A, Giaouris E and Simões M, 2016. Alternative disinfection methods to chlorine for use in the fresh-cut industry. Food Research International, 82, 71–85. https://doi.org/10.1016/j.foodres.2016.01.021

Miceli A and Settanni L, 2019. Influence of agronomic practices and pre-harvest conditions on the attachment and development of *Listeria monocytogenes* in vegetables. Annals of Microbiology, 69, 185–199. https://doi.org/10.1007/s13213-019-1435-6

Moyne AL, Harris LJ and Marco ML, 2013. Assessments of total and viable *Escherichia coli* O157:H7 on field and laboratory grown lettuce. PLOS ONE, 8, e70643. https://doi.org/10.1371/journal.pone.0070643

Mu D-S, Du Z-J, Chen J, Austin B and Zhang X-H, 2021. What do we mean by viability in terms of 'viable but non-culturable' cells? Environmental Microbiology Reports, 13, 248–252. https://doi.org/10.1111/1758-2229.12953

Müller L, Schultz AC, Fonager J, Jensen T, Lisby M, Hindsdal K, Krusell L, Eshøj A, Møller LT, Porsbo LJ, Böttiger BE, Kuhn K, Engberg J and Ethelberg S, 2015. Separate norovirus outbreaks linked to one source of imported frozen raspberries by molecular analysis, Denmark, 2010–2011. Epidemiology and Infection, 143, 2299–2307. https://doi.org/10.1017/s0950268814003409

Müller L, Rasmussen LD, Jensen T, Schultz AC, Kjelsø C, Barnadas C, Sigsgaard K, Larsen AR, Widstrup Jensen C, Jeppesen S, Uhrbrand K, Hove N, Mølbak K and Ethelberg S, 2016. Series of Norovirus outbreaks caused by consumption of green coral lettuce, Denmark, April 2016. PLoS Currents, 8. https://doi.org/10.1371/currents.outbreaks.115761d5d6de6a8bc7dd4b41f0f5f142

Mundi GS, Zytner RG and Warriner K, 2017. Fruit and vegetable wash-water characterization, treatment feasibility study and decision matrices. Canadian Journal of Civil Engineering, 44, 971–983. https://doi.org/10.1139/cjce-2017-0214

Munther D and Wu J, 2013. Enhanced surveillance on food-borne disease outbreaks: dynamics of cross-contamination in biocidal wash procedure. Journal of Theoretical Biology, 321, 28–35. https://doi.org/10.1016/j.jtbi.2012.12.024

Munther D, Luo Y, Wu J, Magpantay FMG and Srinivasan P, 2015. A mathematical model for pathogen cross-contamination dynamics during produce wash. Food Microbiology, 51, 101–107. https://doi.org/10.1016/j.fm.2015.05.010

Murray K, Wu F, Shi J, Jun Xue S and Warriner K, 2017. Challenges in the microbiological food safety of fresh produce: limitations of post-harvest washing and the need for alternative interventions. Food Quality and Safety, 1, 289–301. https://doi.org/10.1093/fqsafe/fyx027

NACMCF (National Advisory Committee on Microbiological Criteria for Foods), 2010. Parameters for determining inoculated pack/challenge study protocols [published correction appears in J Food Prot. 2011 Apr;74(4):522]. Journal of Food Protection, 73, 140–202. https://doi.org/10.4315/0362-028x-73.1.140

Naughton P, Kelly D, Geagan-Murray S, Middleton S, Cosgrove C and Petty-Saphon N, 2021. A food-borne outbreak of cryptosporidiosis likely linked to salad leaves. Irish Medical Journal, 114, 381–390.

Nocker A and Camper AK, 2006. Selective removal of DNA from dead cells of mixed bacterial communities by use of ethidium monoazide. Applied and Environmental Microbiology, 72, 1997–2004. https://doi.org/10.1128/aem.72.3.1997-2004.2006

Oliver JD, 2010. Recent findings on the viable but nonculturable state in pathogenic bacteria. FEMS Microbiology Reviews, 34, 415–425. https://doi.org/10.1111/j.1574-6976.2009.00200.x

Ölmez H and Kretzschmar U, 2009. Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. LWT—Food Science and Technology, 42, 686–693.

Palma-Salgado S, Pearlstein AJ, Luo Y, Park HK and Feng H, 2014. Whole-head washing, prior to cutting, provides sanitization advantages for fresh-cut Iceberg lettuce (*Latuca sativa* L.). International Journal of Food Microbiology, 179, 18–23. https://doi.org/10.1016/j.ijfoodmicro.2014.03.018

Possas A and Pérez-Rodríguez F, 2023. New insights into cross-contamination of fresh-produce. Current Opinion in Food Science, 49, 100954. https://doi.org/10.1016/j.cofs.2022.100954

Pradhan AK, Mishra A and Pang H, 2018. Relevant pathogenic and spoilage microorganisms in vegetables products. In: F Pérez-Rodríguez, P Skandamis and V Valdramidis (eds). Quantitative Methods for Food Safety and Quality in the Vegetable Industry. Food Microbiology and Food Safety, Springer, Switzerland. pp. 29–58.

Qi H, Wang L, Huang Q and Hung Y-C, 2020. Effect of organic load on the efficacy of activated persulfate in inactivating Escherichia coli O157:H7 and the production of halogenated by-products. Food Control, 114, 107218. https://doi.org/10.1016/j.foodcont.2020.107218

Radzevičius A, Dapkienė M, Sabienė N and Dzięcioł J, 2020. A Rapid UV/Vis Spectrophotometric Method for the Water Quality Monitoring at On-Farm Root Vegetable Pack Houses. Applied Sciences, 10.

Raffo A and Paoletti F, 2022. Fresh-cut vegetables processing: environmental sustainability and food safety issues in a comprehensive perspective. Frontiers in Sustainable Food Systems, 5. https://doi.org/10.3389/fsufs.2021.681459

Randtke SJ, 2010. Chemistry of Aqueous Chlorine. White's Handbook of Chlorination and Alternative Disinfectants Fifth Edition, 68–173.

Raymond P, Paul S, Perron A, Bellehumeur C, Larocque É and Charest H, 2022. Detection and sequencing of multiple human Norovirus genotypes from imported frozen raspberries linked to outbreaks in the province of Quebec, Canada, in 2017. Food and Environmental Virology, 14, 40–58. https://doi.org/10.1007/s12560-021-09507-8

Reed E, Ferreira CM, Bell R, Brown EW and Zheng J, 2018. Plant-microbe and abiotic factors influencing *Salmonella* survival and growth on alfalfa sprouts and Swiss chard microgreens. Applied and Environmental Microbiology, 84. https://doi.org/10.1128/aem.02814-17

Riggio GM, Jones SL and Gibson KE, 2019. Risk of human pathogen internalization in leafy vegetables during lab-scale hydroponic cultivation. Horticulturae, 5, 25.

Rimhanen-Finne R, Niskanen T, Lienemann T, Johansson T, Sjöman M, Korhonen T, Guedes S, Kuronen H, Virtanen MJ, Mäkinen J, Jokinen J, Siitonen A and Kuusi M, 2011. A nationwide outbreak of *Salmonella* bovismorbificans associated with sprouted alfalfa seeds in Finland, 2009. Zoonoses Public Health, 58, 589–596. https://doi.org/10.1111/j.1863-2378.2011.01408.x

Rispens JR, Freeland A, Wittry B, Kramer A, Barclay L, Vinjé J, Treffiletti A and Houston K, 2020. Notes from the field: multiple cruise ship outbreaks of Norovirus associated with frozen fruits and berries - United States, 2019. MMWR Morbidity and Mortality Weekly Report, 69, 501–502. https://doi.org/10.15585/mmwr.mm6916a3

Sánchez G, Elizaquível P, Aznar R and Selma MV, 2015. Virucidal effect of high power ultrasound combined with a chemical sanitizer containing peroxyacetic acid for water reconditioning in the fresh-cut industry. Food Control, 52, 126–131. https://doi.org/10.1016/j.foodcont.2014.12.021

Sapers GM, Gorny JR and Yousef AE, 2006. Microbiology of fruits and vegetables. CRC Taylor and Francis Group, Boca Raton, FL.

Sarvikivi E, Roivainen M, Maunula L, Niskanen T, Korhonen T, Lappalainen M and Kuusi M, 2012. Multiple norovirus outbreaks linked to imported frozen raspberries. Epidemiology and Infection, 140, 260–267. https://doi.org/10.1017/s0950268811000379

Saupe AA, Rounds J, Sorenson A, Hedeen N, Bagstad E, Reinberg R, Wagley AG, Cebelinski E and Smith K, 2021. Outbreak of Norovirus gastroenteritis associated with ice cream contaminated by frozen raspberries from China-Minnesota, United States, 2016. Clinical Infectious Diseases, 73, e3701–e3707. https://doi.org/10.1093/cid/ciaa821

Schottroff F, Fröhling A, Zunabovic-Pichler M, Krottenthaler A, Schlüter O and Jäger H, 2018. Sublethal injury and Viable but Non-culturable (VBNC) state in microorganisms during preservation of food and biological materials by non-thermal processes. Frontiers in Microbiology, 9. https://doi.org/10.3389/fmicb.2018.02773

Self JL, Conrad A, Stroika S, Jackson A, Whitlock L, Jackson KA, Beal J, Wellman A, Fatica MK, Bidol S, Huth PP, Hamel M, Franklin K, Tschetter L, Kopko C, Kirsch P, Wise ME and Basler C, 2019. Multistate outbreak of listeriosis associated with packaged leafy green salads, United States and Canada, 2015–2016. Emerging Infectious Diseases, 25, 1461–1468. https://doi.org/10.3201/eid2508.180761

Shaw A, Helterbran K, Evans MR and Currey C, 2016. Growth of *Escherichia coli* O157:H7, Non-O157 Shiga Toxin-Producing *Escherichia coli*, and *Salmonella* in water and hydroponic fertilizer solutions. Journal of Food Protection, 79, 2179–2183. https://doi.org/10.4315/0362-028x.Jfp-16-073

Simons LK and Sanguansri P, 1997. Advances in the washing of minimally processed vegetables. Food Australia, 49, 75–80.

Slayton RB, Turabelidze G, Bennett SD, Schwensohn CA, Yaffee AQ, Khan F, Butler C, Trees E, Ayers TL, Davis ML, Laufer AS, Gladbach S, Williams I and Gieraltowski LB, 2013. Outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 associated with romaine lettuce consumption, 2011. PLOS ONE, 8, e55300. https://doi.org/10.1371/journal.pone.0055300

Smolinski HS, Wang S, Ren L, Chen Y, Kowalcyk B, Thomas E, Doren JV and Ryser ET, 2018. Transfer and Redistribution of *Salmonella* Typhimurium LT2 and *Escherichia coli* O157:H7 during pilot-scale processing of baby spinach, cilantro, and romaine lettuce. Journal of Food Protection, 81, 953–962. https://doi.org/10.4315/0362-028x.Jfp-17-420

Soon JM, Brazier AKM and Wallace CA, 2020. Determining common contributory factors in food safety incidents – a review of global outbreaks and recalls 2008–2018. Trends in Food Science and Technology, 97, 76–87. https://doi.org/10.1016/j.tifs.2019.12.030

Stephan R, Althaus D, Kiefer S, Lehner A, Hatz C, Schmutz C, Jost M, Gerber N, Baumgartner A, Hächler H and Mäusezahl-Feuz M, 2015. Foodborne transmission of *Listeria monocytogenes* via ready-to-eat salad: a nationwide outbreak in Switzerland, 2013–2014. Food Control, 57. https://doi.org/10.1016/j.foodcont.2015.03.034

Teng Z, Van Haute S, Zhou B, Hapeman CJ, Millner PD, Wang Q and Luo Y, 2018. Impacts and interactions of organic compounds with chlorine sanitizer in recirculated and reused produce processing water. PLOS ONE, 13, e0208945. https://doi.org/10.1371/journal.pone.0208945

Toivonen PMA and Lu C, 2013. Differential quenching of free chlorine by organic compounds potentially exuded from injured plant tissues. Postharvest Biology and Technology, 86, 192–194. https://doi.org/10.1016/j.postharvbio.2013.06.035

Truchado P, Gil MI, Kostic T and Allende A, 2016. Optimization and validation of a PMA qPCR method for *Escherichia coli* quantification in primary production. Food Control, 62, 150–156. https://doi.org/10.1016/j.foodcont.2015.10.014

Truchado P, Hernandez N, Gil MI, Ivanek R and Allende A, 2018. Correlation between *E. coli* levels and the presence of food-borne pathogens in surface irrigation water: establishment of a sampling program. Water Research, 128, 226–233. https://doi.org/10.1016/j.watres.2017.10.041

Truchado P, Gil MI, Larrosa M and Allende A, 2020. Detection and quantification methods for Viable but Non-culturable (VBNC) cells in process wash water of fresh-cut produce: industrial validation. Frontiers in Microbiology, 11. https://doi.org/10.3389/fmicb.2020.00673

Truchado P, Gil MI and Allende A, 2021. Peroxyacetic acid and chlorine dioxide unlike chlorine induce viable but non-culturable (VBNC) stage of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in wash water. Food Microbiology, 100, 103866. https://doi.org/10.1016/j.fm.2021.103866

Truchado P, Gómez-Galindo M, Gil MI and Allende A, 2023. Cross-contamination of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in the viable but non-culturable (VBNC) state during washing of leafy greens and the revival during shelf-life. Food Microbiology, 109, 104155. https://doi.org/10.1016/j.fm.2022.104155

Tudela JA, López-Gálvez F, Allende A and Gil MI, 2019a. Chlorination management in commercial fresh produce processing lines. Food Control, 106, 106760. https://doi.org/10.1016/j.foodcont.2019.106760

Tudela JA, López-Gálvez F, Allende A, Hernández N, Andújar S, Marín A, Garrido Y and Gil MI, 2019b. Operational limits of sodium hypochlorite for different fresh produce wash water based on microbial inactivation and disinfection by-products (DBPs). Food Control, 104, 300–307. https://doi.org/10.1016/j.foodcont.2019.05.005

Turner ER, Luo Y and Buchanan RL, 2020. Microgreen nutrition, food safety, and shelf life: a review. Journal of Food Science, 85, 870–882. https://doi.org/10.1111/1750-3841.15049

Tyson RV and Simonne E, 2014. A practical guide for aquaponics as an alternative enterprise. Horticultural Sciences Department, UF/IFAS Extension Available online: http://edis.ifas.ufl.edu

U.S. FDA (U.S. Food and Drug Administration), 2017. Draft guidance for industry: compliance with and recommendations for implementation of the standards for the growing, harvesting, packing, and holding of produce for human consumption for sprout operations. Available online: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/draft-guidance-industry-compliance-and-recommendations-implementation-standards-growing-harvesting

U.S. FDA (U.S. Food and Drug Administration), 2018. Draft guidance for industry: standards for the growing, harvesting, packing, and holding of produce for human consumption. Available online: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/draft-guidance-industry-standards-growing-harvesting-packing-and-holding-produce-human-consumption

Ukuku DO and Sapers GM, 2001. Effect of sanitizer treatments on *Salmonella* Stanley attached to the surface of cantaloupe and cell transfer to fresh-cut tissues during cutting practices. Journal of Food Protection, 64, 1286–1291. https://doi.org/10.4315/0362-028x-64.9.1286

Van Der Linden I, Cottyn B, Uyttendaele M, Vlaemynck G, Maes M and Heyndrickx M, 2014. Evaluation of an attachment assay on lettuce leaves with temperature- and starvation-stressed *Escherichia coli* O157:H7 MB3885. Journal of Food Protection, 77, 549–557. https://doi.org/10.4315/0362-028X.JFP-13-332

Van Haute S, Sampers I, Holvoet K and Uyttendaele M, 2013. Physicochemical quality and chemical safety of chlorine as a reconditioning agent and wash water disinfectant for fresh-cut lettuce washing. Applied and Environmental Microbiology, 79, 2850–2861. https://doi.org/10.1128/aem.03283-12

Van Haute S, Luo Y, Sampers I, Mei L, Teng Z, Zhou B, Bornhorst ER, Wang Q and Millner P, 2018. Can UV absorbance rapidly estimate the chlorine demand in wash water during fresh-cut produce washing processes? Postharvest Biology and Technology, 142, 19–27. https://doi.org/10.1016/j.postharvbio.2018.02.002

Waldram A, Lawler J, Jenkins C, Collins J, Payne M, Aird H, Swindlehurst M, Adak GK, Grant K, Ready D, Gorton R and Foster K, 2018. Large outbreak of multiple gastrointestinal pathogens associated with fresh curry leaves in North East England, 2013. Epidemiology and Infection, 146, 1940–1947. https://doi.org/10.1017/s095026881800225x

Wang Q and Kniel KE, 2016. Survival and transfer of Murine Norovirus within a hydroponic system during kale and mustard microgreen harvesting. Applied and Environmental Microbiology, 82, 705–713. https://doi.org/10.1128/aem.02990-15

Warriner K and Namvar A, 2013. Recent advances in fresh produce post-harvest decontamination technologies to enhance microbiological safety. Stewart Postharvest Review, 9, 1–8. https://doi.org/10.2212/spr.2013.1.3

Wei C and Zhao X, 2018. Induction of Viable but Nonculturable *Escherichia coli* O157:H7 by low temperature and its resuscitation. Frontiers in Microbiology, 9, 2728. https://doi.org/10.3389/fmicb.2018.02728

Weng S, Luo Y, Li J, Zhou B, Jacangelo JG and Schwab KJ, 2016. Assessment and speciation of chlorine demand in fresh-cut produce wash water. Food Control, 60, 543–551. https://doi.org/10.1016/j.foodcont.2015.08.031

Wesche AM, Gurtler JB, Marks BP and Ryser ET, 2009. Stress, sublethal injury, resuscitation, and virulence of bacterial food-borne pathogens. Journal of Food Protection, 72, 1121–1138. https://doi.org/10.4315/0362-028x-72.5.1121

WHO (World Health Organization), 2017. Guidelines for drinking-water quality: fourth edition incorporating the first addendum. World Health Organization, Geneva Licence: CC BY-NC-SA 3.0 IGO.

Williams TR and Marco ML, 2014. Phyllosphere microbiota composition and microbial community transplantation on lettuce plants grown indoors. mBio, 5. https://doi.org/10.1128/mBio.01564-14

Wongkiew S, Hu Z, Lee JW, Chandran K, Nhan HT, Marcelino KR and Khanal SK, 2021. Nitrogen recovery via aquaponics – bioponics: engineering considerations and perspectives. ACS ES&T Engineering, 1, 326–339. https://doi.org/10.1021/acsestengg.0c00196

Wright KM and Holden NJ, 2018. Quantification and colonisation dynamics of *Escherichia coli* O157:H7 inoculation of microgreens species and plant growth substrates. International Journal of Food Microbiology, 273, 1–10. https://doi.org/10.1016/j.ijfoodmicro.2018.02.025

Wu F, Ghamkhar R, Ashton W and Hicks A, 2019. Sustainable seafood and vegetable production: aquaponics as a potential opportunity in urban areas. Integrated Environmental Assessment and Management, 15. https://doi.org/10.1002/ieam.4187

Xiao Z, Bauchan G, Nichols-Russell L, Luo Y, Wang Q and Nou X, 2015. Proliferation of *Escherichia coli* O157:H7 in soil-substitute and hydroponic microgreen production systems. Journal of Food Protection, 78, 1785–1790. https://doi.org/10.4315/0362-028x.Jfp-15-063

Yang M, Cousineau A, Liu X, Luo Y, Sun D, Li S, Gu T, Sun L, Dillow H, Lepine J, Xu M and Zhang B, 2020. Direct metatranscriptome RNA-seq and multiplex RT-PCR amplicon sequencing on nanopore MinION – promising strategies for multiplex identification of viable pathogens in food. Frontiers in Microbiology, 11, 514.

Zhang G, Ma L, Phelan VH and Doyle MP, 2009. Efficacy of antimicrobial agents in lettuce leaf processing water for control of *Escherichia coli* O157:H7. Journal of Food Protection, 72, 1392–1397. https://doi.org/10.4315/0362-028x-72.7.1392

Zhao X, 2023. Food safety hazard identification and characterization of *Bacillus thuringiensis* biopesticides applied on edible plants. PhD dissertation. Falculty of Bioscience Engineering, Ghent, Belgium.

Zhao X, Zhong J, Wei C, Lin CW and Ding T, 2017. Current perspectives on viable but non-culturable state in food-borne pathogens. Frontiers in Microbiology, 8, 580. https://doi.org/10.3389/fmicb.2017.00580

Zhou B, Luo Y, Turner ER, Wang Q and Schneider KR, 2014. Evaluation of current industry practices for maintaining tomato dump tank water quality during packinghouse operations. Journal of Food Processing and Preservation, 38, 2201–2208. https://doi.org/10.1111/jfpp.12200

Zhou B, Luo Y, Nou X, Lyu S and Wang Q, 2015. Inactivation dynamics of *Salmonella enterica, Listeria monocytogenes*, and *Escherichia coli* O157:H7 in wash water during simulated chlorine depletion and replenishment processes. Food Microbiology, 50, 88–96. https://doi.org/10.1016/j.fm.2015.03.004

## Abbreviations

| | |
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| AQ | assessment question |
| CFU | colony forming unit |
| FAO | Food and Agriculture Organization of the United Nations |
| FBO | food-borne outbreaks |
| FBOps | Food Business Operators |
| FDA | U.S. Food and Drug Administration |
| ffFVHS | fresh and frozen fruit, vegetables and herbs |
| FVHs | fruit, vegetables and herbs |
| GAP | Good Agricultural Practices |
| GHPs | Good Hygienic Practices |
| HACCP | Hazard Analysis Critical Control Point |
| ICMSF | International Commission on Microbiological Specifications for Foods |
| OPRP | operational pre-requisite program |
| PAA | peracetic acid or peroxyacetic acid |
| PRP | Pre-requisite programme |
| ROAs | rapid outbreak assessment(s) |
| RTE | ready-to-eat |
| STEC | Shiga toxin-producing *Escherichia coli* |
| SQ | sub question |
| VBNC | viable but non-culturable |
| WHO | World Health Organization |

## Glossary

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| Aeroponics | hydroponic cultivation technique of plants, where plants are growing in the air and the nutrients and water are provided through a mist environment without soil or any aggregate medium. Identified as emerging agricultural practice. |
| Aquaponics (or aquaponic systems) | hydroponic cultivation technique of plants, that uses water from fish farming in an integrated system, identified as emerging agricultural practice. |
| Biocide | a chemical substance or microorganism intended to destroy, deter, render harmless or exert a controlling effect on any harmful organism by chemical or biological means (CX 53-2003).[4] |
| Biocidial product | according to Regulation (EU) No 528/2012[13], 'biocidal product' means: (i) any substance or mixture, in the form in which it is supplied to the user, consisting of, containing or generating one or more active substances, with the intention of destroying, deterring, rendering harmless, preventing the |

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| | action of or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action, (ii) any substance or mixture, generated from substances or mixtures which do not themselves fall under the first indent, to be used with the intention of destroying, deterring, rendering harmless, preventing the action of or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action. A treated article that has a primary biocidal function shall be considered a biocidal product. |
| Blanching | A heat process typically applied to a food for the purpose of inactivating enzymes and/or fixing the product colour (CAC, 1976).[19] |
| Clean water | 'water that does not compromise food safety in the circumstances of its use'. It is clean seawater (natural, artificial or purified seawater or brackish water that does not contain microorganisms, harmful substances or toxic marine plankton in quantities capable of directly or indirectly affecting the health quality of food) and fresh water of a similar quality according to the Regulation (EC) 852/2004[2]. |
| Contamination rate (of process water) | the change (usually increase) per time unit in microbial cell numbers in water mainly due to product entering in contact with process water. The contamination of process water occurs as a result of (i) the transfer of microorganisms detached from ffFVHs surface and soil, debris and dust, (ii) survival against biocide exposure (when used) and (ii) microbial growth in process water depending on the duration of the process and the temperature. However, growth is considered not relevant under most of the continuous processes with periodic or continuous water replenishment compared with the impact of detachment from incoming products. |
| Cross-contamination rate (of product) | The change (usually increase) per unit of time in microbial cell numbers on the ffFVH surface due to re-attachment of cells from process water contaminated from other ffFVH products being previously or simultaneously processed. |
| Fit-for-purpose water: in this opinion used as a synonym of the concept 'process water' | encompassing potable water, clean water, recirculated water or recycled water that could be fit different post-harvest handling and processing operations provided they do not compromise the safety of the final product for the consumer. While water quality will be different in each context, it can be fit to use for certain purposes. Deciding whether water is fit-for-purpose, assessment of the source water, potential hazards linked to this water source, treatment options and their efficacy, multiple barrier processes and the end use of the food product (e.g. if eaten raw) must be considered (FAO and WHO, 2019). |
| Disinfecting | to destroy or irreversibly inactivate specified fungi, bacteria and/or viruses, but not necessarily bacterial spores. |
| Disinfectant | chemical agent or combination of chemical agents that is used on inanimate objects or surfaces. Some chemicals may function as both sanitisers and disinfectants. Disinfectants can be sporostatic but are not necessarily sporicidal. Within the remit of this opinion, disinfectant agents or water disinfection systems are defined as those decontamination agents applied to eliminate microorganisms in process water. |
| Efficacy | the ability to achieve a desired or intended result. In the context of the use of process water, the water management strategies aim to control the water quality to avoid the cross-contamination of the produce. Therefore, a water disinfection treatment will be considered as 'efficacious' when it is able to maintain the microbiological quality of the process water to a level that avoids cross-contamination of ffFVHs during the handling and processing operations. In static conditions, the efficacy of water disinfection treatment could be measured in terms of microbial inactivation (reduction) in the water and/or the lack of cross-contamination between different |

[19] CAC (Codex Alimentarius Commission), 1976. Code of Practice for the processing and handling of quick frozen foods CAC/RCP 8-1976. p. 1–14.

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| | pieces/leaves of ffFVH. However, in dynamic systems (e.g. flume tanks), even if the disinfectant inactivates the microorganisms in the water, no apparent reduction is observed because the microorganisms are periodically transferred to the water with the regular loading of more produce contaminated. In this case, the disinfection treatment is considered efficacious when it avoids the accumulation of microbial indicators and avoid cross-contamination. The threshold/critical limit of microbial indicators that defines the proper quality of the process water is ffFVH and process dependent. |
| Hydroponics | cultivation technique of plants, where plants are growing in the water environment without soil or any aggregate medium. Identified as emerging agricultural practice. |
| Microgreens | Microgreens are young, tender plants that are harvested just after their first true leaves have developed, which is typically around 2–4 weeks after germination. They are often used as a garnish, salad ingredient or as a nutrient-dense addition to a variety of dishes. Microgreens are grown from the same seeds as mature plants, but they are harvested at an earlier stage of growth when the plants are still small and delicate. They are typically grown indoors, in trays or containers, and are often harvested when they are only a few inches tall. |
| Minimal processing | As any action applied to the initial product (e.g. cleaning, coring, peeling, chopping, slicing, washing, dewatering, packaging) and which is not included in the definition of processing from the Regulation (EC) No 852/2004[2]. In this Regulation, 'processing' means any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes. |
| OPRP | OPRP stands for 'Operational Prerequisite Programme(s)' which is defined in the Commission Notice 2022/C 355/01[3] as a control measure or combination of control measures applied to prevent or reduce a significant food safety hazard to an acceptable level and where action criterion and measurement or observation enable effective control of the process and/or product. They are typically linked to the production process and are identified by the hazard analysis as essential, in order to control the likelihood of the introduction, survival and/or proliferation of food safety hazards in the product(s) or in the processing environment. |
| Operational monitoring (on-line, in-line/in-situ, at-line, off-line) | The act of conducting a planned sequence of real-time observations or measurements of control parameters to determine whether a control measure/water management strategy (water disinfection and/or replenishment) is working effectively. More specifically, it refers to procedures required for relevant quality parameter to provide real-time information for a reliable assessment of its status, i.e. providing results with a frequency that enables identifying failures in a timely manner to allow a rapid response (i.e. corrective action to be taken before use of the water supply). Where water is an input to FFV production, routine monitoring of the input water supply and the water quality is required to ensure the water does not compromise the overall safety of the FFV at the point of consumption i.e. it is fit-for-purpose (FAO and WHO, 2021a). |
| Operating procedures/ operating conditions | A set of documented and established steps, rules and regulations that must be followed during the production, processing, handling, and storage of fresh fruits and vegetables. These procedures are put in place to ensure that the produce is safe, fresh and of high quality for consumers to eat. |
| Packing house | Packing houses are an integral part of the fresh produce industry, and are responsible for ensuring that fruits and vegetables are properly prepared and packaged for sale. These facilities are typically located near areas where fruits and vegetables are grown, and are an important part of the agricultural supply chain. The specific operations that take place in a |

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| | packing house can vary depending on the type of fruit or vegetable being processed, but typically include steps such as sort, grade, clean, pack and prepare fresh fruits and vegetables for distribution to retailers or other markets. The goal of these operations is to ensure that the produce is safe, of high quality, free from defects or contaminants, and properly labelled for distribution. |
| Potable water | water that has the drinking water standards, intended for human consumption, according to Directive (EU) 2020/2184[9] of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption. |
| Process water | as a synonym of the concept of 'fit-for-purpose' water, encompasses all types of water that can be used in different post-harvest handling and processing operations, including potable water, clean water, recycled water or recirculated water, knowing that the specific characteristics of process water should be adapted to the specific context and intended use. |
| Recirculated water | Water reused in a closed loop for the same processing operation without replenishment or reconditioning. Example: water in a washing tank that is used to wash large amounts of fruits and vegetables during the same working day (FAO and WHO, 2019). |
| Recycled water | to be used in processing or as an ingredient is not to present a risk of contamination. It is to be of the same standard as potable water, unless the competent authority is satisfied that the quality of the water cannot affect the wholesomeness of the foodstuff in its finished form (Regulation (EC) 852/2004)[2]. Includes: water, other than first-use or reclaimed water, which has been obtained from a processing operation, or water that is reused in the same operation after reconditioning. Examples: water use for transporting or washing of raw materials, such as vegetables and fruits, in subsequent units, for which first-use water is used initially and then reused in previous units until it is used for cleaning of product coming from the field before being discarded or reconditioned (FAO and WHO, 2019). |
| Reused water | includes the definitions of recycled water and recirculated water (FAO and WHO, 2019). |
| Resuscitation | The recovery of a sub-lethally injured bacterium to become fully vital. |
| Sanitising | to reduce microorganisms of public health importance to levels considered safe, based on established parameters, without adversely affecting either the quality of the product or its safety. |
| Sanitiser | typically chemical agents use to reduce, remove or inactive microorganisms. Some chemicals may function as both sanitisers and disinfectants. Within this scientific opinion, sanitisers are defined as decontamination agents applied to reduce the level of microorganisms on the ffFVHs. |
| Urban and peri-urban agriculture (UPA) | FAO and RUAF (2022) have defined urban and peri-urban agriculture as 'the production of food and other outputs and related processes, taking place on land and other spaces within cities and surrounding regions'. |
| Validation | Obtaining evidence that a control measure or combination of control measures, if properly implemented, can control the hazard to a specified outcome. Revalidation may be required in case of changes affecting the control measure. Detailed examples can be found in CAC/GL 69-2008[15], EU Commission Notice, 2022/C355/01[20] and more specifically for water used for fresh produce in FAO and WHO (2021a). |
| Verification | The application of methods, procedures, tests and other evaluations, in addition to monitoring to determine compliance with the HACCP-based procedures, i.e. to determine whether a control measure is or has been operating as intended (EU Commission Notice 2022/C355/01)[20]. Verification |

[20] European Commission, 2022. Commission notice on the implementation of food safety management systems covering Good Hygiene Practices and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses (2022/C 355/01). 16.9.2022, p. 1–58 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52022XC0916(01)

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| | is conducted periodically to demonstrate that the food safety management or water management system is working as planned. Detailed examples can be found in CAC/GL 69-2008[4], EU Commission Notice, 2022/C355/01, and more specifically for water used in for fresh produce in FAO and WHO (2021a). Verification is conducted periodically as part of a FSMS to demonstrate that the applied water management strategies are working effectively, and the process water reached the demanded microbiological quality (defined as fit-for-purpose for the intended use) to avoid cross-contamination of the ffFVHs via the water. |
| Vertical farming | are a novel type of farming in a controlled environment with a total replacement of solar radiation with artificial lighting that provides the necessary nanometres of the spectrum for the growth and development of plants. In vertical farms, plants grow in soilless cultivation systems such as hydroponic, aeroponic (soilless air/mist solution) or even aquaponic systems that allow stacking multiple layers or columns of plants horizontally or vertically. Vertical farms are located in completely isolated spaces from outdoor environment with thermally insulated installations (especially when at the top floor of the building) and airtight structures that give the opportunity to the farmers to control the environment in terms of temperature, humidity and $CO_2$. |
| Water replenishment | The practice of replacing used water with fresh water during the cleaning and rinsing of fresh fruits and vegetables. The frequency of water replenishment during the produce washing process will depend on the volume of produce being washed and the size and type of washing equipment being used. Water refreshment is occasionally used as synonym. |
| Water disinfection treatment | Mechanical, physical, chemical and/or microbiological treatments, including the combinations thereof, that are applied to the water to destroy, inactive, reduce or remove contaminants including microbiological contaminants. |
| Water management strategies | interventions such as water disinfection and/or replenishment treatments or other water treatment techniques. |

# Appendix A – Examples of food categories included under fresh and frozen fruits, vegetables and herbs (adapted from EFSA BIOHAZ Panel, 2013)

**Table A.1:** General and specific food categories included under fresh and frozen fruits, vegetables and herbs

| General food category | Specific food categories |
|---|---|
| **1. Fruit (non-specified)** | |
| **Soft fruits** | **2. Strawberries** |
| | **3. Raspberries** |
| | **4. Other berries** |
| **5. Citrus fruit** | |
| **6. Apples and related fruit** | |
| **7. Stone fruit** | |
| **8. Tropical fruit** | |
| **9. Melons** | |
| **10. Fruit mixes** | |
| **Vegetable fruits** | **11. Tomatoes** |
| | **12. Peppers and aubergines** |
| | **13. Gourds and squashes** |
| | **14. Fresh pods, legumes and grain (only those not dried)** |
| **Leaves** | **15. Leafy greens eaten raw as salads** |
| | **16. Fresh herbs** |
| | **17. Leafy greens mixed with other fresh food of non-animal origin** |
| | **18. Other leaves** |
| **Root and tuberous vegetables** | **19. Carrots** |
| | **20. Other root and tuberous vegetables** |
| **21. Bulb and stem vegetables** | |
| **22. Flowers and flower buds** | |
| **23. Sprouted seeds** | |
| **24. Fungi (mushrooms and yeasts)** | **(only if fresh or frozen)** |
| **25. Sea vegetables** | **(only if fresh)** |

# Appendix B – Search strategies for specific literature reviews for the different AQs and SQs

The combination of search terms (keywords) and Boolean operators to be used for the strings designed for the search strategies for all AQs and SQs are indicated below.

Three databases were considered simultaneously, namely: (i) WoSCC: Web of Science Core Collection, (ii) FSTA: Food Science and Technology Abstracts and (iii) CABI: CAB Abstracts.

As eligibility criteria for all searches the following filters have been applied:

- Language: English.
- Time: 2010–2022 (timepoint when we will run the searches).

The numbers of publications retrieved on 15 February 2022 are indicated under the column "Results".

**SQ1. Which are the relevant combinations of ffFVHS /handling and processing operations requiring the use of water?**

| Set | String for "types of water" | |
|---|---|---|
| #1 | **TOPIC (TS):** "process* water" OR "wash* water" OR "post-harvest* water" OR "post-harvest* water" OR "recycl* water" OR "reus* water" OR "recondition* water" OR "reclaim* water" OR "recirculat* water" | |
| Set | String for "**types of operations**" | |
| #2 | **TOPIC (TS):** washing OR disinfection OR shower* OR spray* OR "ice-cooling" OR "hydrovac-cool*" OR "hydrovac cool*" OR hydrocooling OR "bin dumping" OR "flume recirculation" OR flum* OR transport OR cut* OR peel* OR glaz* OR blanch* OR sorting OR chill* OR rinsing OR shred* OR chop* OR "fresh-cut process*" OR "fresh cut process*" OR "minimal process*" OR cleaning OR pre-washing OR "pre washing" OR "post-harvest handlin" OR "post-harvest handling" OR "post-harvest operation" OR "post-harvest operation" OR "fresh produce process*" OR "post-harvest activit*" OR "post-harvest activit*" | |
| Set | String for "**ffFVHs**" (general designations) | |
| #3 | **TOPIC (TS):** fruit* OR vegetable* OR herb* OR "fresh produc*" OR "frozen produc*" OR microgreens OR "raw vegetable*" OR "raw fruit*" OR "raw produc*" OR ready-to-eat OR "ready to eat" | |
| Set | String for "**ffFVHs**" (categories from Appendix A in draft scientific opinion not mentioned in sets 3 and 4) | |
| #4 | **TOPIC (TS):** strawberr* OR raspberr* OR berr* OR citrus OR apple* OR tomato* OR pepper* OR aubergine* OR gourd* OR squash* OR fresh pod* OR legume* OR pulses OR "leafy green*" OR "food of non-animal origin" OR leave* OR carrot* OR "sprouted seed*" OR sprout* OR mushroom* | |
| Set | String for "**ffFVHs**" (specific examples suggested by WG and from Bt protocol) | |
| #5 | **TOPIC (TS):** lettuce* OR endive OR escarole OR kale OR rocket OR rucola OR spinach OR salad* OR lollo bionda OR lollo rosso OR cabbage OR zucchini OR courgette OR pea OR corn OR leek OR onion* OR pear* OR basil OR chervil OR chive* OR dill OR "lemon verbena" OR marjoram OR mint OR oregano OR parsley OR rosemary OR sage OR sorrel OR tarragon OR thyme OR tuber OR nut OR coconut OR juice OR "leafy brassica" OR fungi OR multileaves OR multileaf | |
| Set | | **Results** |
| #6 | #1 AND #2 AND (#3 OR #4 OR #5) | **62** |

**Eligibility criteria: Publication type = review articles and book chapters**

**SQ2. Which are the most relevant microbiological hazards associated with the previously identified combinations of ffFVHS/handling and processing operations requiring the use of water?**

## SQ2a. First search strategy:

| Set | String for "microbiological hazards-1" | |
|---|---|---|
| #7 | **TOPIC (TS):** microorganism* OR "biological hazard*" OR pathogen OR "pathogenic virus*" OR "enteric virus*" OR "food-borne virus*" OR "food-borne virus*" OR norovirus* OR "Norwalk-like virus*" OR calicivirus* OR "hepatitis A virus" OR HAV OR "hep A" OR "pathogenic bacteri*" OR STEC OR "shiga toxin-producing E. coli" OR EHEC OR "enterohemorrhagic E. coli" OR "enterohemorrhagic Escherichia coli" OR "pathogenic E. coli" OR "pathogenic Escherichia coli" OR listeri* OR salmonell* OR yersini* OR shigella OR campylobacter OR clostridium OR vibrio OR staphylococcus OR bacillus OR aeromonas OR parasi* OR protozoa OR echinococcus OR cryptosporidi* OR giardia OR toxoplasm* OR entamoeba | |
| Set | String for "**association between hazard and relevant combinations of process water/ffFVHs**" | |
| #8 | **TOPIC (TS):** outbreak OR "public health risk" OR surveillance OR monitoring OR incidence OR human cases OR prevalence OR disease OR illness OR recalls OR alert | |
| Set | String for "**microbial hazards-3**" (general designation to cover emerging hazards and including indicators) | |
| #9 | Microorganism* OR "biological hazard*" OR pathogen* OR "pathogenic virus*" OR "pathogenic bacteri*" OR protozoa OR parasit* OR "e. coli" OR "escherichia coli" OR "microbial indicator*" OR Enterobacter* OR bacteriophages OR coliphages OR phages OR "somatic phages" OR "somatic coliphages" OR "F-RNA phages" OR "F-RNA coliphages" OR "total phages" OR "total coliphages" OR "thermotolerant coliforms" OR "fecal coliforms" OR "Listeria spp" OR "fecal indicator bacteria" OR "clostridium spores" OR "clostridium perfringens" OR Enterococci OR "total coliforms" OR "Bacteroides spp" | |
| Set | String for "**model-related words**" | |
| #10 | rate OR transfer OR donor OR recipient OR model OR temperature OR "cross-contaminat*" OR distribution OR *cumulat* OR attach* OR detach* OR translocation OR fraction OR "risk assessment model" OR kinetic* OR "water holding capacity" | |
| Set | **Same search strategy used for SQ2, AQ2, AQ3 and AQ4** | **Results** |
| #11 | (#6 AND #7 AND #8) OR (#6 AND #9 AND #10) | **492** |

**Eligibility criteria: Publication type = review articles and articles**

## SQ2b. Second alternative search strategy

| Set | String for "**ffFVHs**" (general designations) | |
|---|---|---|
| #3 | **TOPIC (TS):** fruit* OR vegetable* OR herb* OR "fresh produc*" OR "frozen produc*" OR microgreens OR "raw vegetable*" OR "raw fruit*" OR "raw produc*" OR ready-to-eat OR "ready to eat" | |
| Set | String for "**ffFVHs**" (categories from Appendix A in draft scientific opinion not mentioned in sets 3 and 4) | |
| #4 | **TOPIC (TS):** strawberr* OR raspberr* OR berr* OR citrus OR apple* OR tomato* OR pepper* OR aubergine* OR gourd* OR squash* OR fresh pod* OR legume* OR pulses OR "leafy green*" OR "food of non-animal origin" OR leave* OR carrot* OR "sprouted seed*" OR sprout* OR mushroom* | |
| Set | String for "**ffFVHs**" (specific examples suggested by WG and from Bt protocol) | |
| #5 | **TOPIC (TS):** lettuce* OR endive OR escarole OR kale OR rocket OR rucola OR spinach OR salad* OR lollo bionda OR lollo rosso OR cabbage OR zucchini OR courgette OR pea OR corn OR leek OR onion* OR pear* OR basil OR chervil OR chive* OR dill OR "lemon verbena" OR marjoram OR mint OR oregano OR parsley OR rosemary OR sage OR sorrel OR tarragon OR thyme OR tuber OR nut OR coconut OR juice OR "leafy brassica" OR fungi OR multileaves OR multileaf | |
| Set | String for "**microbiological hazards-1**" | |
| #7 | **TITLE (TI):** microorganism* OR "biological hazard*" OR pathogen OR "pathogenic virus*" OR "enteric virus*" OR "food-borne virus*" OR "food-borne virus*" OR norovirus* OR "Norwalk-like virus*" OR calicivirus* OR "hepatitis A virus" OR HAV OR "hep A" OR "pathogenic bacteri*" OR STEC OR "shiga toxin-producing E. coli" OR EHEC OR | |

| Set | String for "**ffFVHs**" (general designations) | |
|---|---|---|
| | "enterohemorrhagic E. coli" OR "enterohemorrhagic Escherichia coli" OR "pathogenic E. coli" OR "pathogenic Escherichia coli" OR listeri* OR salmonell* OR yersini* OR shigella OR campylobacter OR clostridium OR vibrio OR staphylococcus OR bacillus OR aeromonas OR parasi* OR protozoa OR echinococcus OR cryptosporidi* OR giardia OR toxoplasm* OR entamoeba | |
| Set | String for "**association between hazard and relevant combinations of process water/ffFVHs**" | |
| #8 | **TITLE (TI):** outbreak OR "public health risk" OR surveillance OR monitoring OR incidence OR human cases OR prevalence OR disease OR illness OR recalls OR alert | |
| Set | | **Results** |
| #11 | (#3 OR #4 OR #5) AND #7 AND #8 | **852** |

**Eligibility criteria: Publication type = articles**

**SQ3. Which are potential emerging microbiological hazards due to emerging agriculture practices in cultivating ffFVHs?**

| Set | String for "**emerging agricultural practices**" | **Results** |
|---|---|---|
| #12 | Aquaponic* OR hydroponics OR aeroponics OR "vertical agriculture" OR vertical farm* OR "mushroom farming" OR (mushroom AND "organic residues") OR (mushroom AND "organic amendments") OR "green farming" OR "urban agriculture" OR microgreens OR "urban agriculture" OR "urban backyard" OR "urban farm*" OR "community agriculture" | |
| Set | String for "**microbial hazards-2**" (general designation to cover emerging hazards) | Results |
| #13 | Microorganism* OR "biological hazard*" OR pathogen* OR "pathogenic virus*" OR "pathogenic bacteri*" OR protozoa OR parasit* | |
| Set | | **Results** |
| #14 | (#3 OR #4 OR #5) AND #12 AND #13 | **87** |

**Eligibility criteria: Publication type = review articles and book chapters**

**SQ4. Which are the potential waterborne (opportunistic) pathogens associated with water sources used in the handling and processing operations of ffFVHs?**

| Set | String for "**water sources**" | |
|---|---|---|
| #15 | "Rain water" OR "borehole water" OR "river water" OR "lake water" OR "municipal water" OR "ground water" OR "recycl* water" OR "reclaim* water" OR "recondition* water" OR "reus* water" OR "surface water" OR groundwater OR "well water" OR "transfer water" OR "water reservoir" OR wastewater OR "recycle* water" OR "recondition* water" OR "recycle* water" OR "treat* water" | |
| Set | String for "**waterborne human pathogens**" | |
| #16 | "waterborne pathogen*" | |
| Set | | **Results** |
| #17 | #15 AND #16 | **294** |

**Eligibility criteria: Publication type = review articles and articles**

**AQ2. What are the routes of contamination for the most relevant microbiological hazards (as identified in AQ 1) in the water used in different post-harvest handling and processing operations for ffFVHS?**

**AQ3. What are the rates of contamination for the most relevant microbiological hazards (as identified in AQ 1) in the water used in different post-harvest handling and processing operations for ffFVHS and between different ffFVHs?**

| Set | Same search strategy used for SQ2, AQ2 and AQ3 | Results |
|---|---|---|
| #11 | (#6 AND #7 AND #8) OR (#6 AND #9 AND #10) | **492** |

**Eligibility criteria: Publication type = review articles and articles**

**Reviewers will separate manually studies according to the study characteristics and design in 3 different groups: 'lab' scale, 'pilot' scale and 'industrial' scale.**

**AQ4. Which good hygiene practices are recommended to ensure appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of ffFVHs? (Preventive measures)**

Focus on guidance docs including from CODEX and FAO/WHO.

**AQ5 (SQ5 and SQ6)**

**SQ5. Which are the most commonly used disinfection treatments for water used during different post-harvest handling and processing operations of ffFVHs by the industry?**

**SQ6. How do the physico-chemical parameters (organic matter (amount and composition), pH, conductivity, etc.) of the process water used during different post-harvest handling and processing operations of ffFVHs affect the efficacy of the most commonly used disinfection treatments identified in SQ5?**

| Set | String for "Desinfection treatments (including parameters)" | |
|---|---|---|
| #18 | Decontamination OR sanitization OR sanitation OR sanitizer OR disinfectant OR disinfection OR "processing aids" OR chlorine OR "sodium hypochlorite" OR ozone OR "chlorine dioxide" OR "peroxyacetic acid" OR "water disinfection treatment" OR "calcium hypochlorite" OR "chlorine derived compounds" OR "free chlorine" OR "total chlorine" OR "sanitized water" OR "decontaminated water" OR "treated water" | |
| Set | | Results |
| #19 | #6 AND #18 | **584** |

**Eligibility criteria: Publication type = review articles and book chapters and articles (for SQ5 and SQ6)**

**AQ6 (SQ8 and SQ9)**

**SQ8. Which is the impact of the different water disinfection treatments on the physiological state of the most relevant microbiological hazards?**

**SQ9. Are VBNC bacterial cells able to recover and/or express virulence in vivo in fresh fruits, vegetables and herbs after washing and during storage?**

| Set | String for "bacterial cell injury" | |
|---|---|---|
| #20 | Injury OR sub-lethal OR "sub lethal" OR inactivation OR reduction OR VBNC OR viable-but-not-culturable OR "viable but not culturable" OR fluorescence OR "flow cytometer" OR "metabolically active" OR viability OR persist* OR activity OR selective OR non-selective OR "non selective" OR growth OR virulence OR virulent OR gene OR "gene expression" OR transcript* OR Caco-2 OR CACO-2 OR invasion OR resuscitation OR recover* OR re-growth OR time-lapse OR cells OR "cell division" OR "damaged bacteria" OR "injured bacteria" OR recovered OR resuscitated OR "culturable and viable bacteria" | |
| Set | | **Results** |
| #21 | #6 AND #18 AND #20 | **436** |

**Eligibility criteria: Publication type = articles**

**AQ7. Which are the most efficacious water replenishment rates (when applicable and/or in combination with disinfection treatments) needed to maintain the appropriate microbiological quality requirements of water used for different post-harvest handling and processing operations of ffFVHs?**

| Set | String for "water replenishment" | |
|---|---|---|
| #22 | "water replenishment" OR "water refresh*" OR "spent water" OR "water refill*" OR "water supply" OR "water renew*" OR "restore water" OR "water recirculate*" | |
| Set | | **Results** |
| #23 | #6 AND #22 | **21** |

**Eligibility criteria: Publication type = articles**

**AQ 8. Which relevant protocols including parameters, analytical methods and frequency can be used to validate and/or verify the appropriate microbiological quality requirements of the water intended to be used for different post-harvest handling and processing operations of ffFVHs?**

**AQ 9. Which relevant protocols including parameters and analytical methods can be used for real-time monitoring of the appropriate microbiological quality requirements of the water intended to be used for different post-harvest handling and processing operations of ffFVHs?**

| Set | String for "validation/verification" | |
|---|---|---|
| #24 | ("process water treatment" OR "process water disinfection treatment*" OR "process parameters" OR indicators OR "microbiological quality" OR procedure OR "sampling plan*" OR "water sampling" OR technology OR method OR "physico-chemical factor" OR "physico-chemical parameter" OR "physicochemical parameter" OR "physicochemical parameter" OR control OR test OR "HACCP water" OR "water management in food business" OR "operational prerequisite*" OR ORP*) AND (Validation OR Verification) | |
| Set | | Results |
| #25 | #6 AND #24 | 35 |
| Set | String for "real-time monitoring" | |
| #26 | ("water quality monitoring programme" OR "water sampling" OR monitoring OR parameter OR indicator OR "critical limit" OR protocol OR online OR on-line OR "on line test*" OR inline OR in-line OR "in line" OR atline OR at-line OR "at line" OR offline OR "off line" OR off-line OR real-time OR "real time" OR sensor OR software OR control OR application OR swab OR "critical control point*" OR CCP) AND monitoring | |
| Set | | Results |
| #27 | #6 AND #26 | 102 |
| Set | | **Results** |
| #28 | #25 OR #27 | **124** |

**Eligibility criteria: Publication type = review articles and book chapters and articles**

## Appendix C – Uncertainty analysis

The sources of uncertainty associated with the available data have been summarised in tabular format (Table C.1), describing the nature or cause of the uncertainties. Additional considerations about the impact of these uncertainties on the conclusions are described.

**Table C.1:** Potential sources of uncertainty linked to the specific AQ

| AQ# or SQ# | Source or location of the uncertainty | Nature or cause of the uncertainty | Impact of the uncertainty on the conclusions |
|---|---|---|---|
| AQ1-AQ2 | Bias in the data retrieved from the reported outbreaks.<br>Incomplete Environmental investigations of outbreaks (root cause investigation). | Reporting differs between and within countries and further between hazards.<br>Frozen products can be investigated at a later stage.<br>Evidence that the processing water is the source of the outbreak. | This uncertainty may lead to underestimation of the relevance of emerging/new hazards, while an overestimation of the relevance of known hazard and product combinations.<br>Frozen products are relatively overestimated as a vehicle compared to fresh produce Water used in postharvest handling and processing operations is not acknowledge as the origin of the contamination.<br>The impact of this uncertainty is classified as moderate. |
| General | Quality of the data extracted from the literature review | Limitations of the available studies and relevant data included in these studies.<br>Variability due to the different experts performing the data extraction. | Regardless of the direction of the impact of these uncertainties on the final conclusion, the experts describe this potential impact as low. |
| General | Quality of data supplied via the EUsurvey questionnaire by the food industry | The questionnaire was limited to the collaborating industries with the WASHTOP consortium.<br>This might lead to bias in the data collection. | Regardless of the direction of the impact of this uncertainty on the final conclusion, the experts describe this potential impact as low. |

# Appendix D – Data reported in the zoonoses database on reported occurrence of strong evidence food-borne outbreaks where ffFVHs were implicated as food vehicle (2014–2020)

**Table D.1:** Reported strong evidence outbreaks associated to ffFVHs in the reporting countries[(a)] in accordance with Directive 2003/99[(b)], 2014–2020

| Food vehicle | Zoonotic agent | Year | Country | Number of | | | |
|---|---|---|---|---|---|---|---|
| | | | | Outbreaks | Human cases | Hospitalisations | Deaths |
| Berries | Norovirus (Norwalk-like virus) | 2016 | Norway | 1 | 14 | 1 | 0 |
| Raspberries | Norovirus (Norwalk-like virus) | 2014 | Sweden | 1 | 23 | 0 | 0 |
| Raspberries | Norovirus (Norwalk-like virus) | 2015 | Sweden | 1 | 65 | 0 | 0 |
| Raspberries (frozen) | Norovirus (Norwalk-like virus) | 2014 | Denmark | 1 | 9 | 0 | 0 |
| Frozen raspberries | Norovirus (Norwalk-like virus) | 2015 | Germany | 1 | 73 | 1 | 0 |
| Frozen raspberries | Norovirus (Norwalk-like virus) | 2016 | Germany | 1 | 25 | 14 | 0 |
| Fresh strawberries | Norovirus (Norwalk-like virus) | 2017 | Finland | 1 | 49 | 0 | 0 |
| Strawberries (frozen) | Norovirus (Norwalk-like virus) | 2014 | Denmark | 1 | 11 | 0 | 0 |
| Strawberries | Virus unspecified - Hepatitis virus - Hepatitis A virus | 2018 | Germany | 1 | 29 | 23 | 0 |
| Frozen berries in red fruit jelly | Norovirus (Norwalk-like virus) | 2014 | Germany | 1 | 240 | 0 | 0 |
| Raspberry | Virus unspecified – Hepatitis virus – Hepatitis A virus | 2020 | Sweden | 1 | 9 | 0 | 0 |
| Contaminated plums | *S.* Enteritidis | 2018 | Serbia | 1 | 6 | 1 | 0 |
| Melon cubes (pre-cut) | *S.* Poona | 2019 | Finland | 1 | 9 | 0 | 0 |
| Imported fresh mint | *S. sonnei* | 2020 | Denmark | 1 | 44 | 13 | 0 |
| Cucumbers | STEC O157 | 2020 | United Kingdom | 1 | 36 | 13 | 0 |
| Zucchini | *S.* Kedougou | 2020 | Finland | 1 | 7 | 2 | 0 |
| Mixed salad | Norovirus (Norwalk-like virus) | 2014 | Sweden | 1 | 138 | 0 | 0 |
| Sprouts, eaten raw in a salad | *S.* Bovismorbificans | 2014 | Germany | 1 | 56 | 13 | 0 |
| Lettuce | *Escherichia coli*, pathogenic – unspecified | 2014 | United Kingdom | 1 | 142 | 14 | 0 |
| Carrots, leeks, lentils | *C. perfringens* | 2014 | United Kingdom | 1 | 14 | 0 | 0 |
| Parsley | *C. parvum* | 2014 | Sweden | 1 | 83 | 0 | 0 |
| Bagged rocket leaves | STEC O157 | 2014 | United Kingdom | 1 | 10 | 2 | 0 |
| Bagged ready to eat salad | STEC O157 | 2014 | United Kingdom | 1 | 102 | 0 | 0 |
| Sweetcorn, peas | *Campylobacter* spp. | 2014 | United Kingdom | 1 | 39 | 0 | 0 |
| Mixed salad | Norovirus (Norwalk-like virus) | 2014 | Germany | 1 | 7 | 0 | 0 |

| Food vehicle | Zoonotic agent | Year | Country | Number of | | | |
|---|---|---|---|---|---|---|---|
| | | | | Outbreaks | Human cases | Hospitalisations | Deaths |
| Salad mix | *Y. enterocolitica* | 2014 | Norway | 1 | 132 | 0 | 0 |
| Sprouts | *S.* Szentes | 2014 | Switzerland | 1 | 11 | 0 | 0 |
| Sprouts | *S.* Bovismorbificans | 2014 | Switzerland | 1 | 23 | 0 | 0 |
| Pre-cut salad | *L. monocytogenes* serovar 4b | 2014 | Switzerland | 1 | 31 | 0 | 4 |
| Coriander | *S. sonnei* | 2015 | Sweden | 1 | 42 | 0 | 0 |
| Frozen onions | *C. perfringens* | 2015 | Germany | 1 | 3 | 0 | 0 |
| Chives | Enterotoxigenic *E. coli* (ETEC) | 2016 | Norway | 1 | 60 | 0 | 0 |
| Salad leaves | STEC O157 | 2016 | United Kingdom | 1 | 170 | 63 | 2 |
| Bean sprouts | *S.* Chester | 2016 | United Kingdom | 1 | 19 | 0 | 0 |
| Mung bean sprouts | *S.* Enteritidis | 2016 | Finland | 1 | 22 | 0 | 0 |
| Rucola | STEC | 2016 | Finland | 1 | 237 | 0 | 0 |
| Salad | *Cryptosporidium parvum* | 2016 | Sweden | 1 | 50 | 0 | 0 |
| Mixed salad | Calicivirus | 2016 | Sweden | 1 | 400 | 0 | 0 |
| Lettuce of the lollo bionda type from 1 to 2 different batches contaminated with Norovirus GI.2 | Norovirus (Norwalk-like virus) | 2016 | Denmark | 1 | 412 | 0 | 0 |
| Mung beans (sprouted) | *S.* Enteritidis | 2017 | Finland | 1 | 32 | 6 | 0 |
| Leaf lettuce | *L. monocytogenes* | 2017 | Switzerland | 1 | 2 | 1 | 0 |
| Salad | Norovirus (Norwalk-like virus) | 2017 | Italy | 1 | 19 | 0 | 0 |
| Bean sprouts | *S.* Chester | 2017 | United Kingdom | 1 | 24 | 4 | 0 |
| Frozen corn | *L. monocytogenes* | 2018 | Finland | 1 | 30 | 30 | 3 |
| Rocket salad leaves | STEC O157 | 2018 | United Kingdom | 1 | 33 | 15 | 0 |
| Cucumber used in ready to eat food products | *S.* Agona | 2018 | United Kingdom | 1 | 76 | 0 | 0 |
| Cucumber salad | *S.* Enteritidis | 2018 | Germany | 1 | 3 | 0 | 0 |
| Frozen sweetcorn | *L. monocytogenes* | 2018 | United Kingdom | 1 | 12 | 12 | 2 |
| Snow peas | *S. sonnei* | 2019 | Norway | 1 | 28 | 0 | 0 |
| Kale | *Cryptosporidium parvum* | 2019 | Sweden | 1 | 20 | 0 | 0 |
| Cherry tomatoes | *S.* Typhimurium, monophasic | 2019 | Sweden | 1 | 82 | 0 | 0 |

| Food vehicle | Zoonotic agent | Year | Country | Number of | | | |
|---|---|---|---|---|---|---|---|
| | | | | Outbreaks | Human cases | Hospitalisations | Deaths |
| Spinach | *Y. enterocolitica* | 2019 | Sweden | 1 | 37 | 0 | 0 |
| Kale | *Cryptosporidium parvum* | 2019 | Sweden | 1 | 32 | 0 | 0 |
| Kale | *Cryptosporidium parvum* | 2019 | Sweden | 1 | 49 | 0 | 0 |
| Kale | *Cryptosporidium parvum* | 2020 | Sweden | 1 | 25 | 0 | 0 |
| Cucumber and tomatoes | *S.* Enteritidis | 2019 | Sweden | 1 | 50 | 0 | 0 |
| Fresh spinach produced by an Italian establishment and packaged at two different locations for the Danish and for the Swedish markets | *Yersinia enterocolitica* biotype 4 | 2019 | Denmark | 1 | 20 | 0 | 0 |

(a): EU countries including Norway and Switzerland. The United Kingdom (of Great Britain and Northern Ireland) withdrew from the EU and became a third country on 1 February 2020 (Agreement on the withdrawal of the United Kingdom of Great Britain and Northern Ireland from the European Union and the European Atomic Energy Community. OJ L 29, 31.1.2020, p. 7). Data from the UK for 2020 were considered non-MS data.

(b): Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. OJ L 325, 12.12.2003, p. 31–40.

# Appendix E – Summary of food-borne outbreak data retrieved from the literature review where ffFVHs were implicated as food vehicle (2010 to 15/2/2022)

**Table E.1:** Summary of food-borne outbreak data retrieved from literature review (2010–2022, until 15 February 2022) where ffFVHs were implicated as food vehicle

| Type of ffFVHs | Zoonotic agent | Food vehicle | Number of outbreaks | Number of human cases | Number of hospitalisations | Number of deaths | Reference |
|---|---|---|---|---|---|---|---|
| **Frozen FVHs** | *Listeria monocytogenes* | Corn | 1 | 47 | NR | 9 | EFSA and ECDC (2018a) |
| | Noroviruses | Raspberries | 1 | 74 | NR | NR | Einöder-Moreno et al. (2016) |
| | | Strawberries | 1 | 10,950 | 38 | NR | Mäde et al. (2013) |
| | | Raspberries | 8 | 242 | NR | NR | Müller et al. (2015) |
| | | Raspberries | 5 | 724 | NR | NR | Raymond et al. (2022) |
| | | Raspberries | 13 | 900 | NR | NR | Sarvikivi et al. (2012) |
| | | Raspberries | 4 | 15 | 1 | NR | Saupe et al. (2021) |
| | | Fruit and berry mix (raspberries) | 3 | 220 | NR | NR | Rispens et al. (2020) |
| **Total** | | | **36** | **13,172** | **39** | **9** | |
| **Fresh-cut FVHs** | *Cryptosporidium parvum* | Frisee salad | 1 | 259 | NR | NR | Åberg et al. (2015) |
| | | Mixed salad | 1 | 300 | NR | NR | McKerr et al. (2015) |
| | | Mixed salad | 1 | 40 | 14 | NR | Naughton et al. (2021) |
| | *Listeria monocytogenes* | Mixed salad | 2 | 33 | 33 | 3 | Self et al. (2019) |
| | | Mixed salad | 1 | 32 | NR | NR | Stephan et al. (2015) |
| | *Salmonella* Newport and Reading | Lettuce | 1 | 106 | NR | 2 | Lienemann et al. (2011) |
| | *Salmonella* Braenderup | Iceberg lettuce | 1 | 116 | 0 | 0 | Gajraj et al. (2012) |
| | *Yersinia enterocolitica* | Spinach | 1 | 57 | NR | NR | Espenhain et al. (2019) |
| | | Mixed salad | 1 | 133 | NR | NR | MacDonald et al. (2016) |
| **Total** | | | **10** | **1,076** | **47** | **5** | |
| **Fresh-whole FVHs** | *Campylobacter jejuni* | Peas | 1 | 43 | NR | NR | Kwan et al. (2014) |
| | *E. coli* (STEC O157) | Leeks, potatoes (soil) | 1 | 252 | 80 | 1 | Launders et al. (2016) |
| | STEC O157 | Lettuce | 1 | 58 | 34 | NR | Slayton et al. (2013) |
| | STEC O145 | Lettuce | 1 | 33 | NR | NR | Baloch (2014) |
| | *Listeria monocytogenes* | Apples | 1 | 3 | 3 | 0 | Marus et al. (2019) |
| | | Stone fruit | 1 | 4 | NR | NR | Chen et al. (2016) |

| Type of ffFVHs | Zoonotic agent | Food vehicle | Number of outbreaks | Number of human cases | Number of hospitalisations | Number of deaths | Reference |
|---|---|---|---|---|---|---|---|
| | Norovirus | Lettuce | 23 | 412 | NR | NR | Müller et al. (2016) |
| | *Salmonella* spp., *E. coli* and *Shigella sonnei* | Curry leaves | 1 | 592 | 2 | NR | Waldram et al. (2018) |
| | *Salmonella* Newport, Hartford and Oranienburg | Sprouted chia | 1 | 31 | NR | NR | Fields et al. (2015) |
| | *Salmonella* Typhimurium | Tomatoes | 1 | 190 | 24 | 0 | Behravesh et al. (2012) |
| | *Salmonella* Newport | Mung bean sprouts | 1 | 126 | 39 | 0 | Bayer et al. (2014) |
| | *Salmonella* Saint Paul | Peppers | 1 | 1,442 | 315 | 2 | Barton Behravesh et al. (2011) |
| | *Salmonella* Typhimurium | Tomatoes | 1 | 82 | NR | NR | Colombe et al. (2019) |
| | *Salmonella* Agona | Cucumber | 1 | 147 | NR | NR | EFSA and ECDC (2018b) |
| | *Salmonella* Havana | Alfalfa sprouts | 1 | 31 | 13 | NR | Harfield et al. (2019) |
| | *Salmonella* Chailey | Coconut | 1 | 19 | NR | NR | Luna et al. (2018) |
| | *Salmonella* Anatum | Peppers | 1 | 32 | 8 | 0 | Hassan et al. (2017) |
| | *Salmonella* Bovismorbificans | Alfalfa seeds | 1 | 42 | NR | NR | Rimhanen-Finne et al. (2011) |
| | *Shigella sonnei* | Basil | 1 | 46 | NR | NR | Guzman-Herrador et al. (2011) |
| **Total** | | | **41** | **3,585** | **518** | **3** | |

NR: not reported.

# Appendix F – Data reported in the zoonoses database on reported occurrence from objective sampling for various microbiological hazards agents in ffFVHs (2014–2020)

**Table F.1:** Reported occurrence from objective sampling for *Campylobacter* spp., *Clostridium* spp., *L. monocytogenes*, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Vibrio* spp., Shiga toxin- producing *E. coli* (STEC), *Yersinia* spp., Hepatitis A virus, Norovirus, *Cryptosporidium* spp., *Giardia* spp., *Toxoplasma* spp. in ffFVHs in the reporting countries in accordance with Directive 2033/99/EC, 2014–2020. (Grey-shaded cells represent sampling stages for which no occurrence data has been reported for the respective microbiological hazard.)

| Sampling stage | Zoonotic agent | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Campylobacter* spp. | | | *Clostridium* spp. | | | *L. monocytogenes* | | | *Salmonella* spp. | | |
| | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) |
| **Farm** | 6 | 0 | 0.00 | 1 | 0 | 0.00% | 992 | 0 | 0.00 | 2,971 | 3 | 0.10 |
| **Processing plant** | 207 | 0 | 0.00 | | | | 5,199 | 37 | 0.71 | 16,660 | 51 | 0.31 |
| **Packing centre** | | | | | | | 66 | 1 | 1.52 | 103 | 1 | 0.97 |
| **Retail** | 5,776 | 8 | 0.14 | 48 | 2 | 4.17% | 18,680 | 213 | 1.14 | 63,176 | 344 | 0.54 |
| **Wholesale** | 313 | 0 | 0.00 | | | | 594 | 2 | 0.34 | 3,119 | 22 | 0.71 |
| **Mobile retailer or market/ street vendor** | | | | | | | | | | | | |
| **Catering** | | | | | | | 1,222 | 4 | 0.33 | 4,499 | 6 | 0.13 |
| **Restaurant or Cafe or Pub or Bar or Hotel or Catering service** | 131 | 0 | 0.00 | 24 | 0 | 0.00 | 1,045 | 3 | 0.29 | 4,102 | 6 | 0.15 |
| **School or kindergarten** | | | | | | | 3 | 0 | 0.00 | 4 | 0 | 0.00 |

| Sampling stage | Zoonotic agent | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Campylobacter* spp. | | | *Clostridium* spp. | | | *L. monocytogenes* | | | *Salmonella* spp. | | |
| | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) |
| **Take-away or fast-food outlet** | | | | | | | | | | 1 | 0 | 0.00 |
| **Hospital or medical care facility** | | | | | | | 36 | 0 | 0.00 | 87 | 0 | 0.00 |
| **TOTALS** | **6,433** | **8** | **0.12** | **73** | **2** | **2.74** | **27,837** | **260** | **0.93** | **94,722** | **433** | **0.46** |

| Sampling stage | Zoonotic agent | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Shigella* spp. | | | *Staphylococcus* spp. | | | *Vibrio* spp. | | | STEC | | |
| | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) |
| **Farm** | | | | | | | | | | 670 | 0 | 0.00 |
| **Processing plant** | | | | 3 | 0 | 0.00 | | | | 2,244 | 2 | 0.09 |
| **Packing centre** | | | | | | | | | | | | |
| **Retail** | 36 | 0 | 0.00 | 29 | 0 | 0.00 | 198 | 0 | 0.00 | 11,943 | 19 | 0.16 |
| **Wholesale** | | | | | | | 19 | 0 | 0.00 | 1,342 | 0 | 0.00 |
| **Mobile retailer or market/ street vendor** | | | | | | | | | | | | |
| **Catering** | | | | 179 | 1 | 0.56 | | | | 84 | 0 | 0.00 |

| Sampling stage | Zoonotic agent | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Shigella* spp. | | | *Staphylococcus* spp. | | | *Vibrio* spp. | | | STEC | | |
| | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) |
| **Restaurant or Cafe or Pub or Bar or Hotel or Catering service** | 84 | 0 | 0.00 | 67 | 0 | 0.00 | | | | 389 | 0 | 0.00 |
| **School or kindergarten** | | | | | | | | | | | | |
| **Take-away or fast-food outlet** | | | | | | | | | | | | |
| **Hospital or medical care facility** | | | | 14 | 0 | 0.00 | | | | | | |
| **TOTALS** | **120** | **0** | **0.00** | **292** | **1** | **0.34** | **217** | **0** | **0.00** | **16,672** | **21** | **0.12** |

| Sampling stage | Zoonotic agent | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Yersinia* spp. | | | Hepatitis A virus | | | Norovirus | | | *Cryptosporidium* spp. | | |
| | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) |
| **Farm** | 2 | 0 | 0.00 | | | | | | | | | |
| **Processing plant** | 65 | 0 | 0.00 | 9 | 0 | 0.00 | 9 | 0 | 0.00% | | | |
| **Packing centre** | | | | | | | | | | | | |
| **Retail** | 106 | 0 | 0.00 | 181 | 0 | 0.00 | 214 | 1 | 0.47% | 31 | 0 | 0.00 |
| **Wholesale** | 1 | 0 | 0.00 | 2 | 0 | 0.00 | 3 | 0 | 0.00% | | | |

| Sampling stage | Zoonotic agent | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Yersinia* spp. | | | Hepatitis A virus | | | Norovirus | | | *Cryptosporidium* spp. | | |
| | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) |
| **Mobile retailer or market/ street vendor** | | | | | | | | | | | | |
| **Catering** | 1 | 0 | 0.00 | | | | | | | | | |
| **Restaurant or Cafe or Pub or Bar or Hotel or Catering service** | 5 | 0 | 0.00 | 84 | 0 | 0.00 | 97 | 0 | 0.00% | | | |
| **School or kindergarten** | | | | | | | | | | | | |
| **Take-away or fast-food outlet** | | | | | | | | | | | | |
| **Hospital or medical care facility** | | | | | | | | | | | | |
| **TOTALS** | **180** | **0** | **0** | **276** | **0** | **0** | **323** | **1** | **0.31** | **31** | **0** | **0.00%** |

| Sampling stage | Zoonotic agent | | | | | |
|---|---|---|---|---|---|---|
| | *Giardia* spp. | | | *Toxoplasma* spp. | | |
| | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) |
| **Farm** | | | | | | |
| **Processing plant** | | | | 3 | 0 | 0.00% |
| **Packing centre** | | | | | | |
| **Retail** | 31 | 0 | 0.00% | 29 | 0 | 0.00% |

| Sampling stage | Zoonotic agent | | | | | |
|---|---|---|---|---|---|---|
| | *Giardia* spp. | | | *Toxoplasma* spp. | | |
| | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) | Sampling units tested | Positive sampling units (n) | Positive sampling units (%) |
| **Wholesale** | | | | | | |
| **Mobile retailer or market/street vendor** | | | | | | |
| **Catering** | | | | 179 | 1 | 0.56% |
| **Restaurant or Cafe or Pub or Bar or Hotel or Catering service** | | | | 67 | 0 | 0.00% |
| **School or kindergarten** | | | | | | |
| **Take-away or fast-food outlet** | | | | | | |
| **Hospital or medical care facility** | | | | 14 | 0 | 0.00% |
| **TOTALS** | **31** | **0** | **0.00** | **292** | **1** | **0.34** |

## Annex A – Protocol for the mandate on microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVHs)

Annex A is available under the Supporting Information section on the online version of the scientific output.

## Annex B – Industry survey on current industrial practices on fresh and frozen fruits, vegetables and herbs (ffFVHs) and the post-harvest handling and processing operations where water is used

Annex B is available under the Supporting Information section on the online version of the scientific output.