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SCIENTIFIC OPINION

Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVH). Part 4 (fresh-cut FVH process water management plan)

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

Water used in post-harvest handling and processing operations is an important risk factor for microbiological cross-contamination of fruits, vegetables and herbs (FVH). Industrial data indicated that the fresh-cut FVH sector is characterised by process water at cooled temperature, operational cycles between 1 and 15 h, and product volumes between 700 and 3000 kg. Intervention strategies were based on water disinfection treatments mostly using chlorine-based disinfectants. Water replenishment was not observed within studied industries. The industrial data, which included 19 scenarios were used to develop a guidance for a water management plan (WMP) for the fresh-cut FVH sector. A WMP aims to maintain the fit-for-purpose microbiological quality of the process water and consists of: (a) identification of microbial hazards and hazardous events linked to process water; (b) establishment of the relationship between microbiological and physico-chemical parameters; (c) description of preventive measures; (d) description of intervention measures, including their validation, operational monitoring and verification; and (e) record keeping and trend analysis. A predictive model was used to simulate water management outcomes, highlighting the need for water disinfection treatments to maintain the microbiological quality of the process water and the added value of water replenishment. Relying solely on water replenishment (at realistic feasible rates) does not avoid microbial accumulation in the water. Operational monitoring of the physico-chemical parameters ensures that the disinfection systems are operating effectively. Verification includes microbiological analysis of the process water linked to the operational monitoring outcomes of physico-chemical parameters. Although *Escherichia coli* and *Listeria* spp. could be indicators for assessing water quality, food business operators should set up and validate a tailored WMP to identify physico-chemical parameters, as well as microbial indicators and their threshold levels, as performance standards for maintaining the fit-for-purpose microbiological quality of the process water during post-harvest handling and processing operations.

KEYWORDS

cutting, fit-for-purpose water, industry, operational monitoring, validation, verification, wash water, water disinfection, water quality, water safety

CONTENTS

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 (ffFVH), to provide guidance on the use of water in the production of ffFVH and to describe 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 ffFVH and the routes and rates of contamination of the water and the ffFVH; (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 ffFVH, 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 ffFVH.

The mandate includes five outputs (scientific opinions). The already published Part 1 Opinion (EFSA BIOHAZ Panel, 2023) contains the literature review and analysis of the outbreak data and stakeholder questionnaire replies. The Part 2 Opinion contains a summary of the development of a dynamic mass balance model for processing operations using water in ffFVH. Parts 3, 4 and 5 Opinions focus specifically on the fresh-whole, fresh-cut and frozen FVH sectors, respectively. This Part 4 opinion is specific to the fresh-cut FVH sector with an emphasis on data generated from the EFSA outsourced activities described in Gil et al. (2025). These data were analysed to understand the industrial practices followed by the industrial collaborators included in this tender, and the relevant outputs were used to address the TORs.

First, the SO describes the post-harvest handling and processing operations using water and highlights the main characteristics of the sector. Based on the data obtained from the food business operators (FBOps) (EFSA outsourced activities – Gil et al., 2025), the main characteristics of the sector were identified. Results showed that the water temperature was usually controlled and cooled (6–11°C). The operational cycle duration varied between a few hours and up to 15 h, and the amount of product processed during the operation cycles varied between 300 and 3000 kg. Water volumes of the water tanks varied widely between 800 and 3000 L. The product-to-water contact time during operations was between 0.5 and 5 min. Still, in most cases, very short contact times occurred (i.e. less than 1 min).

Second, this SO addresses the main components of a water management plan (WMP) aiming to maintain a fit-for-purpose microbiological quality of process water. The WMP covers (a) the identification of microbial hazards and hazardous events linked to process water; (b) the establishment of relationships between microbial and physico-chemical parameters of the process water; (c) a description of preventive measures; (d) a description of intervention measures, including their validation, operational monitoring and verification; and (e) record keeping and trend analysis. There are common aspects applicable to the three FVH sectors included in specific scientific Opinions Parts 3, 4 and 5.

Across the three sectors, certain microorganisms – such as *Listeria monocytogenes*, *Salmonella* spp. and pathogenic *Escherichia coli* – are consistently identified as the most important microbiological hazards, particularly in cases where water is repeatedly reused or insufficiently disinfected. Common hazardous events for all the sectors include (i) an incomplete removal of contaminated water and/or inadequate cleaning and disinfection between operations and (ii) using the same water to wash large volumes of product during an operational cycle without a well-managed intervention strategy.

In order to analyse the microbiological data of process water obtained from industrial scenarios (EFSA outsourced activities – Gil et al., 2025), three layers of analyses were performed: (i) graphical representation (box-plot) of the levels of potential microbial indicators for process water samples in which pathogens were either detected or not detected, allowing calculation of the percentage of observations exceeding different thresholds of the potential microbial indicators; (ii) calculation of the odds ratio (OR) of detecting pathogens in relation to different thresholds of potential microbial indicators, aggregating data for sampling visit; and (iii) multivariable logistic mixed-effect modelling using the entire data set to assess the effect of microbial indicator levels on pathogen detection, accounting for the hierarchical structure of the data set. Statistically significant ORs were found in all the sectors, suggesting potential useful threshold levels for some microbial indicators. However, this logistic model revealed that pathogen detection in process water samples is influenced by multiple factors – such as the specific FVH product, type of operation and operational conditions – introducing a substantial random effect tied to each specific scenario for pathogen detection. The complexity of the data set (EFSA outsourced activities – Gil et al., 2025), which was not initially generated to establish associations between microbial indicators and pathogens, further complicated the identification of relevant microbial indicators and setting of thresholds that could reliably predict pathogen detection in process water. Therefore, the suitability of any microbial indicator for verification purposes within a WMP should be validated under the specific operational conditions of each FBOp.

Preventive measures aim to minimise the microbiological contamination in process water during post-harvest handling and processing operations. These measures are primarily based on Good Hygiene Practices (GHPs) and Good Manufacturing Practices (GMPs), which help maintain water quality throughout handling and processing. The key preventive measures include (a) infrastructure and fit-for-purpose buildings and equipment, (b) cleaning and disinfection of equipment and the environment, (c) technical maintenance and calibration, (d) water and air quality control, (e) personnel management and (f) working methodology. These preventive measures will help to establish a basic level of process water quality control before further interventions are implemented.

A primary intervention measure commonly used was the application of chemical disinfectants. Chlorine-based treatments, peroxyacetic acid (PAA) and hydrogen peroxide (H_2O_2) were commonly applied by the industry. An effective disinfectant application to the water requires real-time monitoring of different parameters such as residual disinfectant levels, pH and other physico-chemical parameters. No water replenishment practices were observed in any industrial scenario across the sectors; instead, smaller volume refilling strategies were used to sustain constant water levels. In the fresh-cut FVH sector, intervention strategies were based on water disinfection treatments using chlorine-based disinfectants, followed by PAA and electrolysed water.

In order to assist FBOps in implementing optimal intervention strategies and understanding the impact of these on process water quality, simulations of the effect of hypothetical scenarios representative of each sector (EFSA outsourced activities – Gil et al., 2025) were carried out through a mathematical model described in the Part 2 Opinion (EFSA BIOHAZ Panel, 2025a) and made available as a user-friendly tool (https://r4eu.efsa.europa.eu/app/WaterManage4You). Model simulations indicated that effective water management using chlorine-based disinfectants requires continuous operational monitoring and adjustment of physico-chemical parameters, such as the disinfectant residual levels and pH, to maintain process water microbiological quality within acceptable ranges. Conversely, the simulations showed that using water replenishment (at realistic feasible rates) alone as an intervention strategy was insufficient to maintain a fit-for-purpose microbiological quality. A combination of water disinfection and replenishment provided a more effective water management strategy.

Each FBOp should conduct a validation study to assess the efficacy of intervention measures, which will also support the selection of physico-chemical parameters (e.g. residual disinfectant, pH) as well as of the specific microbial indicators and corresponding thresholds (performance standards) to be used in the operational monitoring and verification procedures, respectively. This study should account for the fit-for-purpose water concept, tailored to the specific handling and processing operations, variability in operating conditions and the intended use of FVH, among other factors.

Specifically for the fresh-cut FVH sector, the most relevant microorganisms identified as hazards in process water included *Salmonella* spp. and Shiga toxin-producing *E. coli* (STEC). *L. monocytogenes* and *E. coli* O157:H7 were not detected. In addition to the common hazardous events listed above, an additional hazardous event included nutrient-rich water due to the release of nutrients from cut FVH.

A relationship was observed between the detection of enteric pathogens and high levels of microbial indicators using industrial data collected through EFSA outsourced activities. When microbial indicators exceeded defined thresholds (e.g. TBC > 10^5 CFU/100 mL; TC > 10^4 CFU/100 mL or *E. coli* or *Listeria* spp. counted above 1 CFU/100 mL), the odds of detecting enteric pathogens like *Salmonella* spp. were more than 100-fold the odds for microbial indicators below these thresholds. However, the above-mentioned logistic regression model showed that the detection of *Salmonella* spp. in process water is an event influenced by multiple factors/variables (e.g. a specific combination of FVH product, type of operation, operational conditions), which leads to an important random effect mainly linked to the scenario. Therefore, the suitability of any potential microbial indicator for verification purposes within the WMP should be validated under the specific operational conditions of each FBOp.

It is recommended that relevant stakeholders use the developed mathematical model for their FVH sector to understand the impact of certain parameters and intervention measures on the process water quality, using specific data generated in their industrial settings. Evaluation of potential chemical hazards associated with the use of water disinfectants was outside the remit of this opinion. However, these need to be assessed in a WMP and linked to the fit-for-purpose microbiological quality of the post-harvest process water to be used.

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 Felício 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 foodborne outbreaks in Europe (Machado-Moreira et al., 2019). Moreover, frozen vegetables and fruit have also been 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., 2021).

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, 2014b, 2014c, 2014d, 2014e, 2020). Water use during harvesting and processing has been identified as an important risk factor for contamination of fruits, vegetables and herbs (FVH). 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 (ffFVH) (EFSA BIOHAZ Panel, 2014a). The process water used after blanching vegetables in the deep-freezing industry is also important (EFSA BIOHAZ Panel, 2020). The microbiological quality of the water that comes into contact with ffFVH is an important consideration and should be controlled by an operational prerequisite program (oPRP) to avoid cross-contamination (EFSA BIOHAZ Panel, 2020; FAO/WHO, 2019).

Large volumes of water are used during harvest and post-harvest handling and processing operations (e.g. washing, rinsing, the use of a flume, 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 ffFVH. Therefore, most post-harvest processors favour 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 large volumes of ffFVH may be processed. Hence, organic matter, microorganisms, including pathogens, and chemical residues can accumulate in the water, thus causing cross-contamination between batches, which is a major concern (FAO/WHO, 2019). The quality of water used in post-harvest handling practices and during processing operations of ffFVH should be monitored and controlled to avoid an accumulation of microbiological hazards.

Most current recommendations specify that post-harvest water that comes in contact with ffFVH 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/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.

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 (FAO/WHO, 2019; Gombas et al., 2017), if water is contaminated during washing and then used to process large quantities of ffFVH, it can be a vehicle for cross-contamination.

In order 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 adversely affect the quality and safety of the produce. Therefore, regardless of the wash method used, growers and processors should follow good practices that ensure and maintain appropriate water quality.

[1]Council Directive 98/83/EEC of 3 November 1998 on the quality of water intended for human consumption (OJ L 330, 5.12.1998, pp. 32–54) has been replaced by 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) (OJ L 435, 23.12.2020, pp. 1–62).

[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, pp. 1–23.

[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, pp. 1–40.

National rules within Member States exist and may create trade barriers since some prohibit using water disinfection treatments in the 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 the maintenance of the microbiological quality of process water as well as the application of water disinfection treatments by the food business operators (FBOps). The proper operation of water disinfection treatment (e.g. application rate, in-use concentration and residual concentration on ffFVH), as well as monitoring the efficacy, has to be conducted properly and safely. As established by FAO/WHO (2019), water quality must be maintained throughout the processing operation, and special attention must be paid to common wash and flume systems and reused water.

Water quality and use in post-harvest handling and processing operations are an increasing concern at the 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 43rd 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 (ffFVH) to provide guidance on the use of water in the production of ffFVH, 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 aims to describe the microbiological hazards associated with the use of water in post-harvest handling and processing operations of ffFVH and the routes and rates of contamination of the water and the ffFVH.

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 ffFVH?

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 ffFVH?

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 ffFVH batches during different post-harvest handling and processing operations using the same water?

TOR2 aims 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 ffFVH, 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 ffFVH?

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 ffFVH?

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 ffFVH?

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 ffFVH?

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

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 ffFVH?

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 ffFVH?

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

1.2 | Additional information

The Mandate on Microbiological Hazards in Water Use during Post-harvest Operations of Fresh and Frozen Fruits, Vegetables and Herbs (ffFVH) is a self-task mandate from the BIOHAZ Panel, including multiple outputs. It integrates a work package that consists of outsourced activities, including tasks such as literature reviews, experimental data collection in industrial settings and modelling, as detailed in the external scientific report (Gil et al., 2025).

The mandate includes five outputs (scientific opinions), as illustrated in Figure 1. The already published Part 1 opinion (EFSA BIOHAZ Panel, 2023) contains the literature review and analysis of the outbreak data and stakeholder questionnaire. The Part 2 Opinion contains a summary of the development of a dynamic mass balance model for processing operations using water in ffFVH. Parts 3, 4 and 5 Opinions focus specifically on the fresh-whole, fresh-cut and frozen FVH sectors, respectively. The same approach and structure are used for each sector-specific opinion (Parts 3–5), aiming to produce concise opinions offering sector-specific guidance. This is achieved by extracting information from the experimental data generated through EFSA's outsourced activities coupled with modelling based on these outcomes. A user-friendly tool has been also developed to allow FBOps to analyse their data and use predictive mathematical modelling to understand the impact of their intervention measures on microbial indicator levels (https://r4eu.efsa.europa.eu/app/WaterManage4You).

This opinion only covers some of the sub-TORs from TOR1 (i.e. TOR 1.1), mostly because industrial data were unavailable to provide further knowledge apart from what has been already included in the Part 1 Opinion. However all the sub TORs from TOR 2 and TOR 3 were addressed. Throughout the text, all the opinions from this mandate will be referred to as 'Part 1 opinion', 'Part 2 opinion', etc.

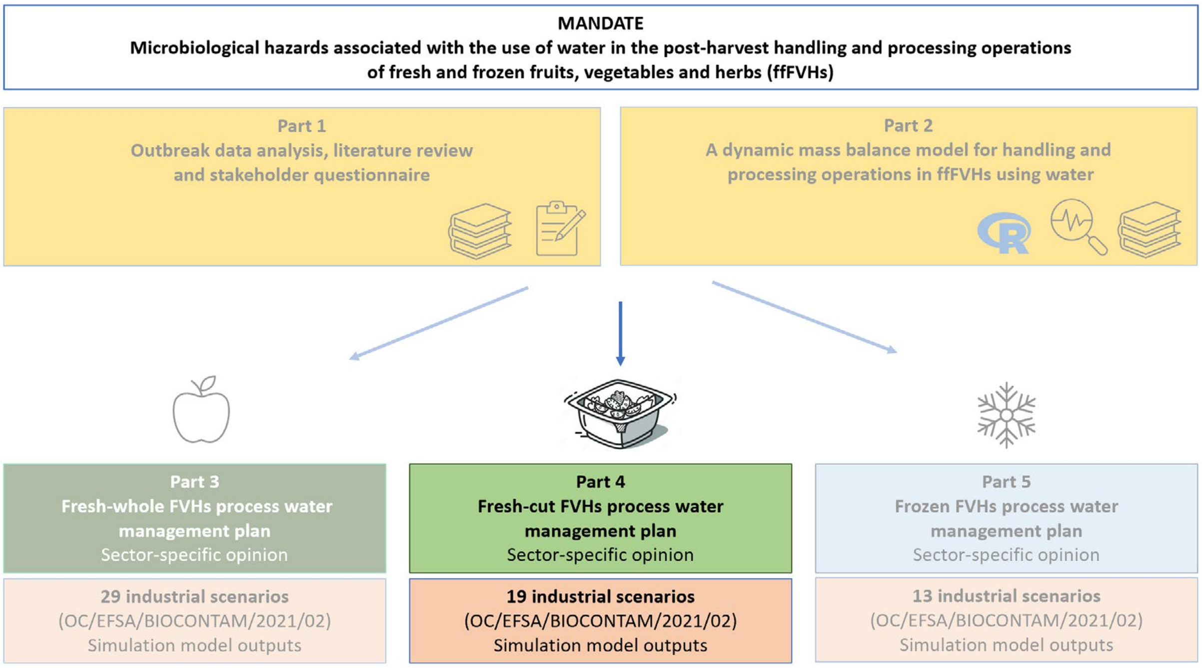

FIGURE 1 Outputs from the mandate on 'Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables, and herbs (ffFVH)' (including EFSA outsourced activities – Gil et al., 2025).

1.3 | Interpretation of the Terms of Reference

This scientific opinion for the fresh-cut FVH sector focuses on the evaluation of microbiological hazards that might be present in the process water used in different handling and processing operations. Chemical hazards are out of the remit of this Scientific Opinion. The aim is to identify the main microbiological hazards and understand the routes of contamination of the process water used in different operations of the fresh-cut FVH, together with the strategies that can be applied by FBOps to keep the microbiological quality of the process water according to its use, i.e. fit-for-purpose quality.[5] The transfer of microorganisms from the process water to the product as well as from product to product (ToR 1.3) is out of the remit of this opinion.

The fresh-cut FVH sector-specific guidance focuses on all the TORs previously described in the Part 1 Opinion (EFSA BIOHAZ Panel, 2023), with an emphasis on evidence generated from the EFSA outsourced activities described in Gil

[5]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 for 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).

et al. (2025). This data set was analysed to understand the industrial practices followed by the industrial collaborators included in this tender, and the relevant outputs were used to address the TORs.

Based on these findings, it is important to define the following terms when referring to the process water management:

(1) **Refilling**: Adding a small amount of water (e.g. potable water, process water) during the operations to maintain a constant volume in the water tank/equipment/process lines. In this case, the water added during the refilling replaces the water lost from the water tank by the movement of the product or due to spilling during the process. This volume is not enough to dilute the organic matter and microorganisms accumulated in the process water due to the introduction of FVH. Therefore, it is not considered as a water management intervention strategy. Based on the data obtained from the industrial settings sampled in the context of EFSA's outsourced activities (Gil et al., 2025), the volumes added during refilling are usually small (< 100 L/h in a 2500 L tank). This agrees with available information (Barrera et al., 2012; Gil et al., 2016). Moreover, the volume of water added is not usually monitored by the FBOps.

(2) **Replenishment (or Refreshment)**: In the Part 1 Opinion of this mandate (EFSA BIOHAZ Panel, 2023), water replenishment was defined as the 'practice of replacing used water with fresh water during the cleaning and rinsing of fresh fruits and vegetables'. Water replenishment was considered as a potential intervention strategy to dilute the organic matter and the microorganisms accumulated in the water tank. To achieve a diluting effect of organic matter and microorganisms in the process water due to the water replenishment strategy, the volume of water added to the water tank should be considerably high (e.g. ≥ 50% of the total volume, 1 m^3/h in a 2500 L water tank) (Allende et al., 2016; Gil et al., 2016). Based on the information retrieved from the industrial settings sampled in the context of EFSA's outsourced activities (Gil et al., 2025), water replenishment is currently not performed by the fresh-cut FVH sector. Only water refilling is done as described above.

(3) **Complete removal**: In the fresh-cut FVH sector, after a certain time, the water tank/equipment/processing lines are (almost) fully emptied and the tank is filled again with new (fresh) water. This means that almost all the water (e.g. 90% or more water from a tank) present in the water tank is removed and replaced by new fresh water (e.g. municipal tap water, reconditioned water).

Data from the industrial settings sampled in the context of EFSA's outsourced activities ($n = 61$, considering the 3 sectors for fresh-whole, fresh-cut and frozen FVH) were obtained from two distinct sampling visits. In each sampling visit, six sampling time points were selected within one operational cycle, and duplicate samples were collected.

The working group defined the 'operational cycle' as the period between (almost completely) filling and emptying the water tank used for the handling and/or processing operation. Sampling time points were distributed from the start of the operation (process start), generally it coincided with the filling of the water tank which is the start of the operational cycle, but it was not always possible. In some cases, the operation may begin sometime after the tank is filled with water, and emptying may occur after the completion of the sampling and handling activities. It should be noted that if the filling and emptying times of the water tank were unknown or not applicable, the operational cycle was considered to be the time between the start and end of the handling process.

In the fresh-cut FVH sector, the operational cycle duration ranged from 5 to 15 h. For shorter cycles, samples were taken at regular intervals, such as every hour for a 5-h operational cycle (e.g. at 0, 1, 2, 3, 4 and 5 h). For longer cycles, sampling time points were adjusted accordingly to ensure that data was collected more or less evenly distributed throughout the entire process. Overall, the sampling time points were strategically distributed to capture changes in water quality throughout a significant portion of the operational cycle, ensuring a comprehensive assessment.

2 | DATA AND METHODOLOGIES

2.1 | Data

2.1.1 | Literature review

The information retrieved from the literature searches for the Part 1 scientific opinion was used in this sector-specific opinion. Details of the methodology followed for the literature search can be found in EFSA BIOHAZ Panel (2023).

2.1.2 | Data collection

The external scientific report describing EFSA's outsourced activities contains data representative of the fresh-cut FVH industry settings. It is relevant to address some of the specific assessment questions in this mandate. The main objective of this tender was to gain insights into the characteristics of the process water and practices followed by the industry to maintain water quality used during the post-harvest handling and processing operations for ffFVH. The case studies (scenarios) selected by the tenderer included three types of ffFVH: (i) fresh-whole FVH, (ii) fresh-cut FVH and (iii) frozen FVH. Data include the characterisation of the water used in different post-harvest handling and processing operations of ffFVH with the aim of evaluating the microbiological and physico-chemical quality of the process water in industry settings. Several physico-chemical parameters were included in the assessment (water temperature, pH, oxidation–reduction potential (ORP), electrical conductivity (EC), residual concentration of disinfectant, total chlorine, total dissolved solids (TDS), turbidity, total soluble solids (TSS), chemical oxygen demand (COD), unfiltered and filtered UV-absorbance, redox potential). The assessment of the microbiological quality of the process water included enumeration of total bacterial count (TBC), total coliforms (TC), *E. coli, Listeria* spp., moulds and yeasts, F-specific phages, total phages and human gut-associated DNA bacteriophage named crAssphage. However, bacteriophages were only determined in selected samples. Detection of foodborne pathogens was also performed, including *Salmonella* spp., *L. monocytogenes*, Shiga toxin-producing *Escherichia coli* (STEC) (including O157:H7), norovirus (GI and GII) and *Cryptosporidium* spp. Levels of VBNC cells were determined in selected samples, including TBC, TC, *E. coli* and *Listeria* spp., as well as the capsid integrity of norovirus. Spores of *C. perfringens* have also been analysed in specific samples. In this scientific opinion, data obtained for the fresh-cut FVH sector were considered. Not all the data generated in EFSA's outsourced activities has been used in the assessment of this scientific opinion. The most relevant data was selected based on the objective of the analyses. The results of all the analyses performed during the two sampling visits to each FBOp are included in Annex A which contains all the data collected.

2.2 | Methodologies

Data collected for fresh-cut FVH from EFSA's outsourced activities were used to answer the different TORs. Data were compiled in an Excel file, including information related to the characterisation of the post-harvest handling and/or processing operations (e.g. volume of product, volume of water, characteristics of the process water, type of intervention) and the results regarding the physico-chemical parameters and microbiological analysis obtained in different sampling points and visits to the industries covering different scenarios.

2.2.1 | Data analysis

Data regarding the outputs of the handling and processing operations generated by EFSA's outsourced activities (Gil et al., 2025), which included 19 industrial scenarios of the fresh-cut FVH sector, were analysed in three steps.

First, results of the levels of potential microbial indicators (TBC, TC, *E. coli* and *Listeria* spp.) (CFU/100 mL) corresponding to each individual sample were $\log_{10}$ transformed and plotted in figures to facilitate the exploratory analysis.

In addition, the counts of potential microbial indicators (TBC, TC, *E. coli* and *Listeria* spp.) for samples in which enteric pathogens (agglutination-confirmed *Salmonella* and/or PCR-confirmed STEC) were detected and not detected were graphically represented through box-and-wisher plot using R (version 4.3.2) and R Studio (version 2023.2.3.561). Graphical representation included the median, interquartile range (IQR as boxes) and min–max range (as whisker). Potential outlier dots included values out of 1.5 times the IQR below Q1 and above Q3. Results of potential microbial indicators below their respective LOD were set as 0 $\log_{10}$ CFU/100 mL by setting these results as 0.01 CFU/mL and then transformed into 1 CFU/100 mL. Thresholds (in $\log_{10}$ scale) for microbial indicators were defined and used to compute the percentage of observations with microbial indicator levels exceeding these thresholds, which could be considered as potential process water performance standards for verification purposes. This was done using all the results of analysed process water samples for the fresh-cut FVH sector.

TABLE 1 Relevant thresholds selected for the potential microbial indicator groups TBC, TC, *E. coli* and *Listeria* spp.

Indicator	Levels of microbial indicator
TBC	4, 5 and 6 $\log_{10}$ CFU/100 mL (10^4, 10^5, 10^6 CFU/100 mL)
TC	2, 3 and 4 $\log_{10}$ CFU/100 mL (10^2, 10^3, 10^4 CFU/100 mL)
E. coli	0, 1 and 2 $\log_{10}$ CFU/100 mL (1, 10 and 100 CFU/100 mL)
Listeria spp.	0, 1 and 2 $\log_{10}$ CFU/100 mL (1, 10 and 100 CFU/100 mL)

Abbreviations: CFU, colony forming unit; TBC, total bacterial count; TC, total coliforms.

Secondly, the relationship between the occurrence of potential microbial indicators above the thresholds indicated in Table 1 and the detection of enteric pathogens (agglutination-confirmed *Salmonella* spp. and/or PCR-confirmed STEC)[6] (as an outcome) was explored by aggregating observations within each sampling visit. In the aggregated data, the pathogen was considered detected if it was identified at least once in any of the sampling points and replicated samples within each visit. The odds ratio[7] (OR) of detecting enteric pathogens depending on the threshold (Th) of the potential microbial indicator (MI) was calculated as:

$$\mathrm{OR} = \frac{\mathrm{Odds\ (MI \geq Th)}}{\mathrm{Odds\ (MI < Th)}} = \frac{a/b}{c/d},$$

[6]48 out of 50 detected enteric pathogens were *Salmonella* spp.-positive samples, with all 48 agglutination-confirmed.

[7]Odds measure how likely the detection of the pathogen is, and is calculated when the indicator is below and above the given threshold.

where

a is the number of visits in which the pathogens were **detected** in the process water and the level of the microbial indicator was **equal to or above** the potential threshold at least in one of the samples of process water analysed within the operational cycle.

b is the number of visits in which the pathogens were **never detected** in the process water and the level of the microbial indicator was **equal to or above** the potential threshold at least in one of the samples of process water analysed within the operational cycle.

c is the number of visits in which the pathogens were **detected** in the process water and the level of the microbial indicator was **below** the potential threshold in all the samples of process water analysed within the operational cycle.

d is the number of visits in which the pathogens were **never detected** in the process water and the level of the microbial indicator was **below** the potential threshold in all the samples of process water analysed within the operational cycle.

The OR confidence interval at 95% and the statistical significance (*p* value) of the *z* statistic were calculated with MedCalc[8] for the three different thresholds defined above for TBC, TC, *E. coli* or *Listeria* spp. as potential microbial indicators.

It is worth-noting that the OR is equivalent to the exponential of the regression parameter of a univariable logistic model that includes a single microbial indicator group as an explanatory variable, dichotomised according to each of the three thresholds as 'below/above' and the probability of detection of pathogens (logit-transformed) as dependent variable. An OR greater than one suggests increased odds of detection of the pathogen when the indicator exceeds the selected threshold.

Thirdly, a multivariable logistic mixed effect model was developed with the entire data set to assess the potential effect of the levels of the potential microbial indicators (in $\log_{10}$ CFU/100 mL) on the detection of the relevant pathogen (*Salmonella*) in process water. Food sectors included in the model for *Salmonella* were only fresh-cut FVH and frozen FVH since no positive results were observed in fresh-whole FVH. The hierarchical structure in the data was accounted for considering nested random factors (i.e. (i) the scenarios, (ii) the sampling visits nested into scenarios and (iii) sampling time points nested into sampling visits and scenarios). To reduce the complexity of the model, the replicates effect was not considered, i.e. the microbial indicator levels of the two sample replicates of each sampling time point were averaged (before Log-transformation), and the pathogen detection was determined as positive if detected in at least one of the two replicates. The interactive effects of the microbial indicator levels by food sectors were included as fixed factors. Only a subset of the four microbial indicators was included in each model to avoid multicollinearity of the explanatory variables.

Two different models for *Salmonella* were set and compared for their ability to fit the data. The complete statistical analysis performed, including exploratory analysis and the modelling, is described in Annex B.

In order to identify appropriate physico-chemical parameters, the industrial data were used to detect potential relationships between the physico-chemical parameters and microbiological parameters. A principal component analysis (PCA) was performed among microbiological and physico-chemical parameters characteristics of process water using the JMP Pro 15.0.0 (390308) (SAS Institute Inc., Cary, NC, USA). The analysis aimed to reduce data dimensionality while preserving essential information, enabling, if possible, the identification of underlying patterns and relationships between the variables.

2.2.2 | Identification of intervention measures

The dynamics of the effect of the different intervention measures applied in the different post-harvest handling and/or processing operations were addressed by consulting modelling approaches described in the literature. Despite the available information, none of the existing models could readily simulate the contamination/inactivation dynamics of all possible post-harvest handling and/or processing operations. As such, substantial amendments and customizations in the model structures and assumptions were required to tailor the models to different processes at an industrial scale. The model described in the Part 2 Model opinion, which was developed within EFSA's outsourced activities was fed with data obtained in industrial cases from the fresh-cut FVH sector to simulate different scenarios, which mimic situations observed in the sampled industrial settings (Gil et al., 2025). A user-friendly tool allowing the simulation of different scenarios of intervention strategies (e.g. water replenishment and water disinfection treatments) as well as the management of these aiming to avoid cross-contamination of FVH by process water has also been developed (https://r4eu.efsa.europa.eu/app/WaterManage4You).

2.3 | Uncertainty analysis

As recommended by the EFSA guidance and related principles and methods on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018a, 2018b), an uncertainty analysis was implemented. Given the narrative nature and context of the TORs of the mandate, which do not include any assessment request, the uncertainty analysis was restricted to an overview of the uncertainty sources affecting the different TORs/AQs (Table A1 in Appendix A).

[8]MedCalc Software Ltd. Odds ratio calculator. https://www.medcalc.org/calc/odds_ratio.php (Version 22.023; accessed April 7, 2024).

3 | ASSESSMENT (FRESH-CUT FVH PROCESS WATER MANAGEMENT PLAN)

3.1 | Post-harvest handling and processing operations using water

3.1.1 | Flow charts of the processing lines and identification of water sources

Data generated by EFSA's outsourced activities include industrial scenarios for the following types of fresh-cut FVH: shredded carrots, curly endive and radicchio, baby leaves, parsley, salad mix with carrots, tomatoes/cucumbers, diced onions, carrot sticks, fresh-cut lettuce, shredded lettuce and salad mix (Gil et al., 2025) (Appendix B). These scenarios correspond to products that, in most cases, are sold as ready-to-eat products. This means that the processing steps included in the processing lines of fresh-cut FVH are those necessary to provide the consumer with a convenient product ready to be consumed. This is the main difference with the activities included in the other two sectors (i.e. fresh-whole FVH and frozen FVH), where some products can be sold as ready-to-eat, while other products are meant to be cooked or peeled before consumption. It should be noted that in cases such as baby leaves or radicchio, the product is not cut before washing or packaging, which means that some products included within the fresh-cut FVH sector are not necessarily subjected to cutting, mostly due to their size, which is already appropriate for consumption as a final ready-to-eat product.

Handing and processing operations using water for fresh-cut FVH have been extensively described in the Part 1 opinion. Figure 2 shows a general flow chart for the handling and processing of fresh-cut FVH, which includes steps where water can be used, like cutting/trimming/dicing/peeling, (pre-)washing and rinsing (Appendix B). It is relevant to mention that frequently, process water was sampled during washing after an initial pre-washing (scenario IDs 31, ID 36, ID 37, ID 38, ID 40, ID 41, ID 42, ID 44, ID 45, ID 46 and ID 47), while in other cases water was sampled from the pre-washing (just after cutting) or washing step. In some cases, water was taken from the rinsing step, which usually takes place after washing (scenario ID 46). However, in one scenario (ID 42), water was taken from the rinsing step that took place between the pre-washing and washing steps.

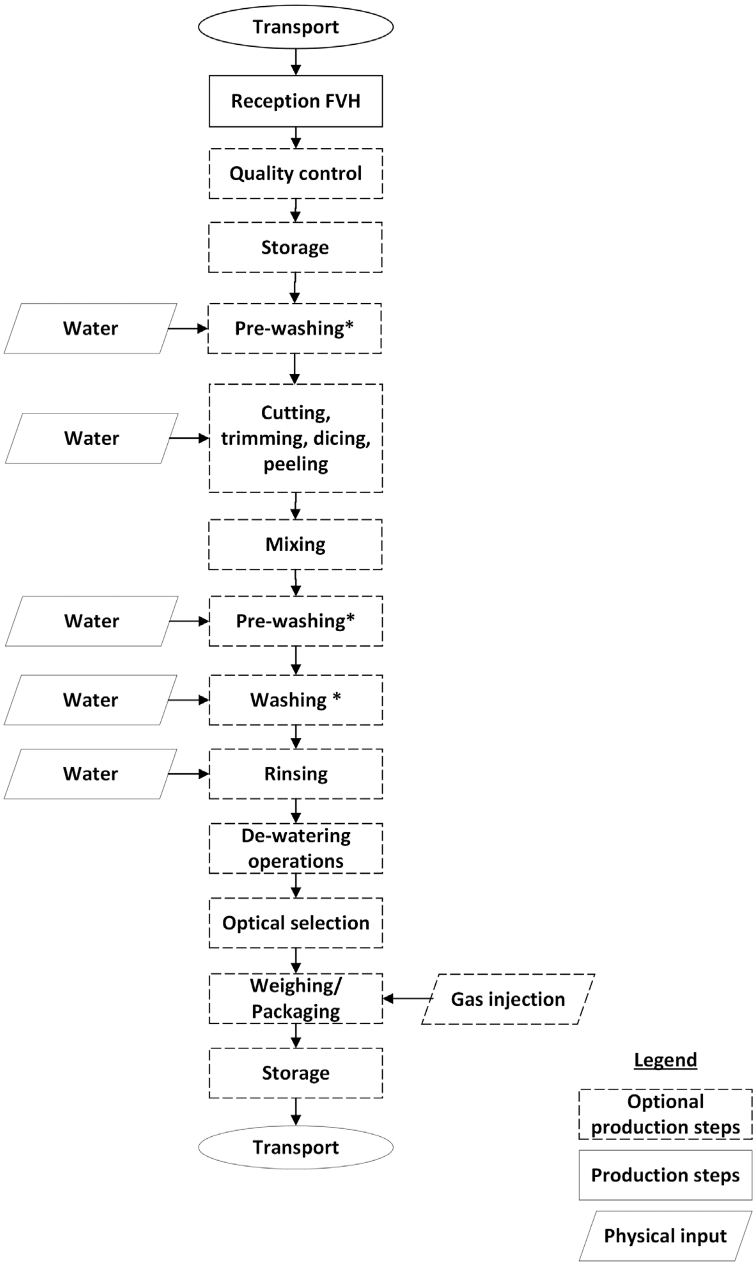

FIGURE 2 A general flow chart for handling and processing fresh-cut fruits, vegetables and herbs (FVH), which includes steps where water can be used. The flow chart can vary between FBOps (e.g. following order of steps, optional steps present or not) and represents a general description of the post-harvest processing and handling operations. *Production steps sampled during EFSA's outsourced activities (Gil et al., 2025).

3.1.2 | Characterisation of the industrial handling and processing operations for the fresh-cut FVH sector

Data generated by EFSA's outsourced activities included a total of 19 industrial scenarios for fresh-cut FVH processing (Gil et al., 2025). Municipal tap water is the main water source used to fill the tank (13 scenarios). However, in 6 scenarios, well water or well water combined with municipal water have been used (Appendix B).

Five scenarios did not apply any intervention strategy aiming to maintain the microbiological quality of the process water. Scenarios lacking any intervention strategy typically employed municipal tap or well water for washing operations involving leafy greens (such as curly endive and radicchio, baby leaves, parsley and a salad mix with carrots) as well as shredded carrots (Appendix B). Fourteen scenarios used different water disinfection treatments, such as sodium and calcium hypochlorite, peroxyacetic acid (PAA) and electrolysed water. Electrolysed water is commonly generated by an electrochemical cell through the electrolysis process. An electrochemical cell setup usually includes an electrolyte solution (containing water and dissolved sodium chloride), electrodes (anode and cathode) and a power source. When an electric current is passed through the saline solution, several oxidation and reduction reactions occur at the electrodes, which generate chlorine gas and, therefore, free chlorine (FC), as well as several reactive oxygen species (ROS). Due to the difficulty of quantifying ROS, the residual concentration of the water disinfection treatment in the case of electrolysed water is usually determined as FC. The operational cycle duration varied among the different case studies. Some operational cycles lasted about 1.3 h, while in other cases, these lasted 15 h.

Figure 3 shows the accumulated mass of fresh-cut FVH processed during an operational cycle. Differences were observed among scenarios, with a maximum amount of product being processed of about 11.2 tonnes in about 6.5 h. Among all the scenarios, leafy greens are the most predominant commodity with 13 scenarios, followed by 4 scenarios of bulbs and roots and 2 scenarios of vegetable fruits.

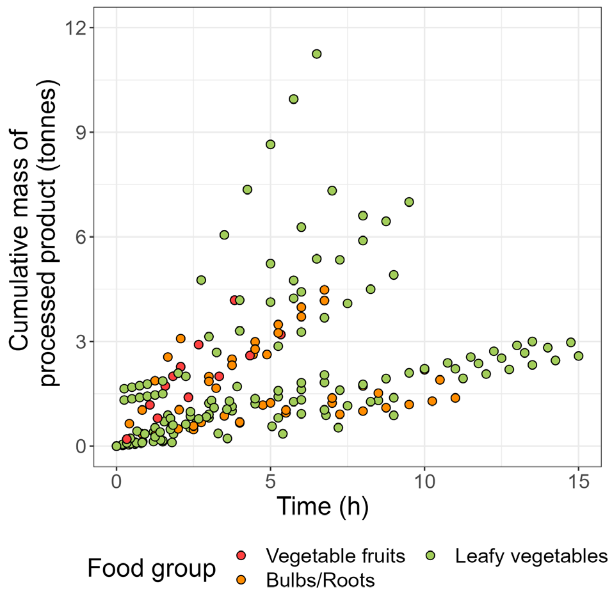

FIGURE 3 Cumulative mass of the product (tonnes) being processed during each sampling time point of the operational cycle. *Source*: EFSA outsourced activities (Gil et al., 2025).

Despite the large mass of product being processed (Figure 3), most of the fresh-cut FVH FBOps only reported a minimum partial refilling of the water tank with unknown volumes of water. Based on the physico-chemical and microbiological characteristics of the process water during the production, it could be concluded that water replenishment (or refreshment) is not applied as an intervention strategy (EFSA outsourced activities – Gil et al., 2025). FBOps only refill the water tank to maintain the volume of water in the tank during the operational cycle.

Figure 4 shows the results of the measured water temperature and COD of process water of fresh-cut FVH along the operational cycle in scenarios with no water treatment and in those using chlorine-based and PAA disinfectants.

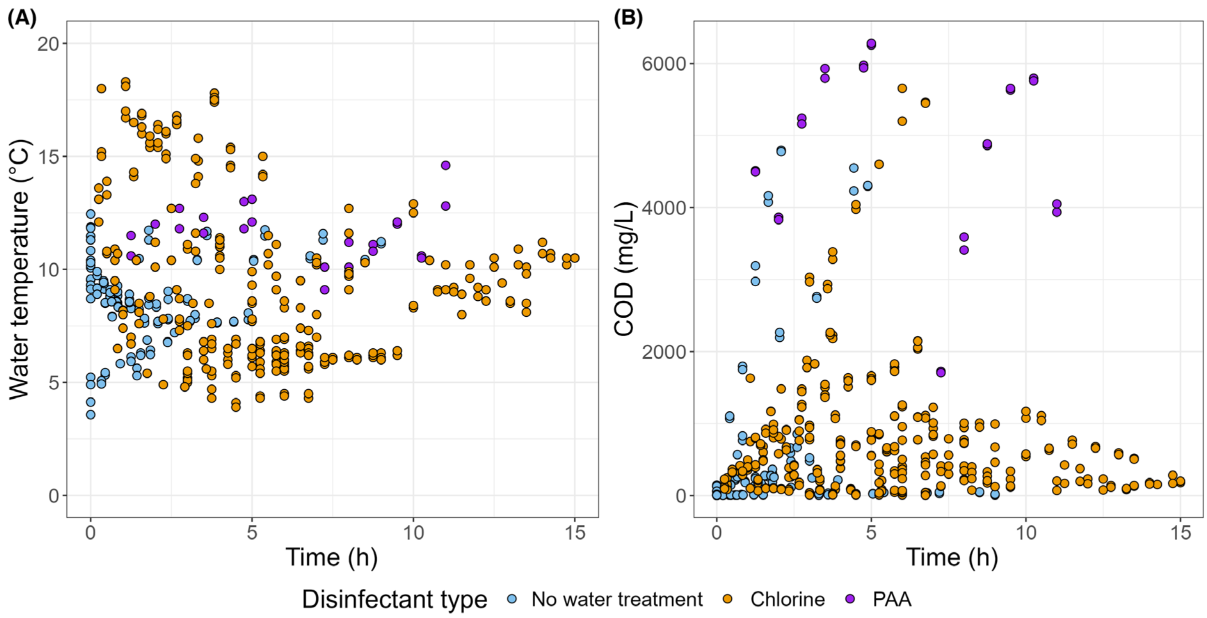

FIGURE 4 Water temperature (°C) (A) and chemical oxygen demand (COD, mg/L) (B) of the process water applied for fresh-cut FVH during each operational cycle. PAA, peroxyacetic acid. *Source*: EFSA outsourced activities (Gil et al., 2025).

One characteristic of the fresh-cut FVH sector is that, in most cases, the temperature of the process water is usually cooled and varied between 3–5°C and up to 18°C (Figure 4A). In most of the cases (75%), the water temperature was between 6 and 11°C. Cooled conditions are recommended to keep the quality of the product as well as to avoid microbial growth. Specific guidelines for the fresh-cut FVH sector recommend keeping the water temperature around 3–4°C (EFSA BIOHAZ Panel, 2023).

The COD, which illustrates the oxygen consumption resulting from the chemical oxidation of organic matter accumulated in the process water, showed differences among the different scenarios (Figure 4B). COD concentrations showed dramatic rises from 2.3 to 4795 mg/L, particularly notable in shredded carrots (scenario ID 30). In most of the cases (75%), COD concentrations were between 230 and 1100 mg/L. Increases in the COD values of process water can be associated with different factors, including (1) the large mass of product being washed, (2) only using refilling, (3) the release of nutrients after cutting, as well as (4) the type of water disinfection treatment applied. For instance, the use of PAA significantly increases the COD of the water (EFSA BIOHAZ Panel, 2023). Adding PAA, an organic acid, to the process water increases the organic load of the process water. Therefore, in most of the cases, those scenarios where PAA is applied showed the highest COD values (e.g. scenario ID 38). Detailed information related to the industrial data can be found in Gil et al. (2025).

The pH of process water was, in most of the cases (75%), between 7.3 and 8.3. (Figure 5A). These cases included scenarios with and without water disinfectant treatment. The lowest pH values (pH ≤ 6) belonged to those cases using PAA as water disinfectant at a concentration between 20 and 90 mg/L. The addition of PAA, an organic acid, considerably reduces the pH of the process water (pH around 5). Based on the recommendations found in the scientific literature (EFSA BIOHAZ Panel, 2023), the pH of process water should be maintained between 6.0 and 6.5 when chlorine-derived compounds, such as sodium or calcium hypochlorite, are applied to ensure that the disinfectant (free chlorine, FC) is in its active form (hypochlorous acid). In those scenarios where water disinfectants were used, the residual concentration was very variable (Figure 5B). When chlorine-based disinfectants were used, FC concentrations varied from 0 to 275 mg/L, while in the case of PAA (scenario ID 38), residual concentrations varied from 20 to close to 90 mg/L. In the case of electrolysed water, residual FC concentrations changed from less than 1 to 74 mg/L. Detailed information related to the industrial data can be found in Gil et al. (2025).

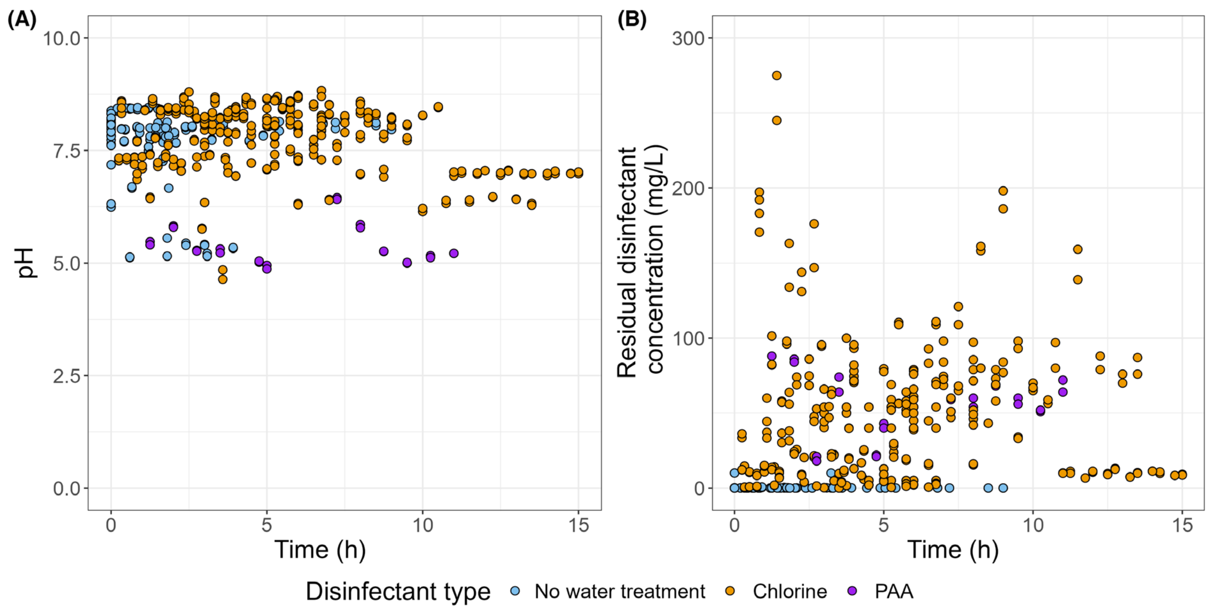

FIGURE 5 pH (A) and residual concentration of disinfectant (mg/L) (B) of the process water applied for fresh-cut FVH during each operational cycle. PAA, peroxyacetic acid. *Source*: EFSA outsourced activities (Gil et al., 2025).

The volumes of water applied were very variable (Figure 6A). The water tanks included in the fresh-cut FVH scenarios had a water volume between 800 and 3000 L. The contact times between water and product were also variable, ranging from 30 s to up to 4 min. However, contact times were mostly below 1 min (Figure 6B). Detailed information related to the industrial data can be found in Gil et al. (2025).

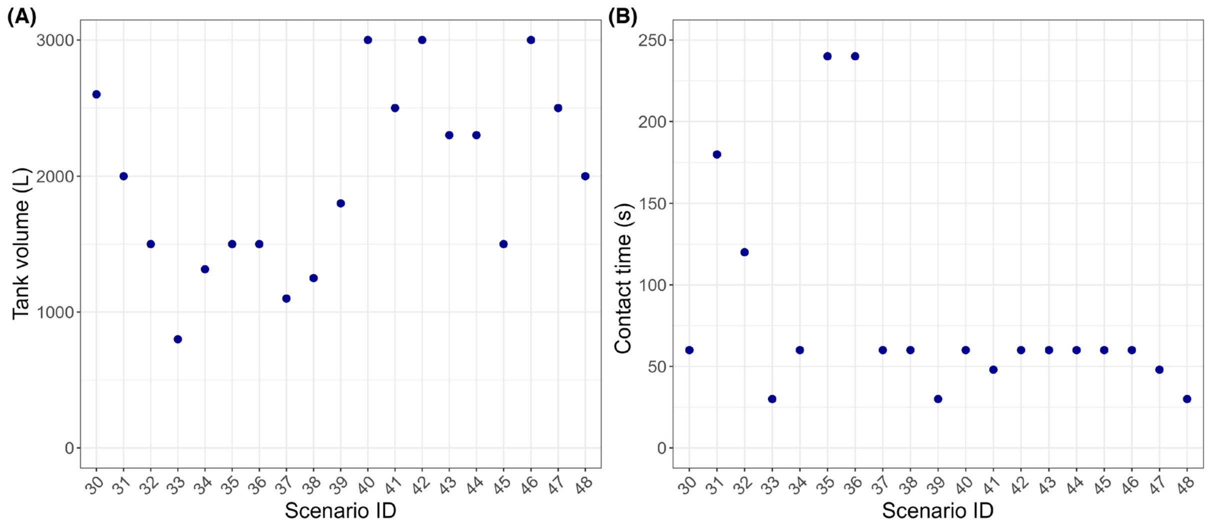

FIGURE 6 Tank volume (L) (A) and contact time (seconds) between product and water (B) in the process water samples from the fresh-cut FVH scenarios ID 30 to ID 48 during each operational cycle. *Source*: EFSA outsourced activities (Gil et al., 2025).

The microbial accumulation in the process water during processing and handling operations of the fresh-cut FVH sector is represented in Figure 7.

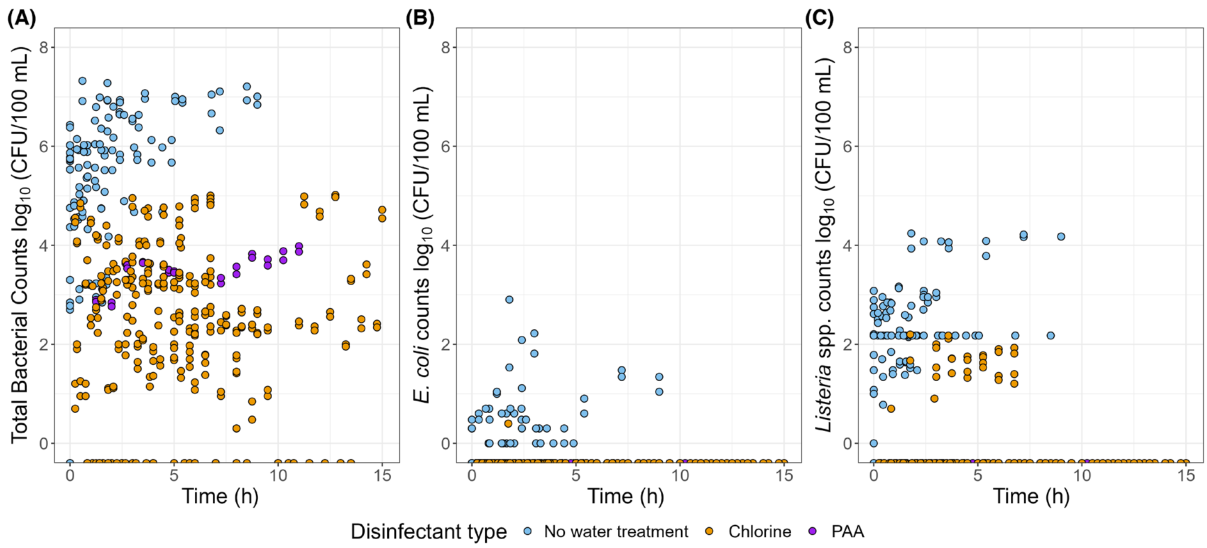

FIGURE 7 Distribution of total bacterial counts (A), *E. coli* (B) and *Listeria* spp. counts (C) (in log_{10} (CFU/100 mL)) in process water treated with chlorine, PAA or without water treatment in industrial scenarios during each operational cycle of the fresh-cut FVH. Bacterial counts for the three microbial groups (A–C) below the respective limit of detection (LOD) are represented below the '0' value in the Y-axis to distinguish these results from the real '0' observations (i.e. 1 CFU/100 mL for *E. coli* and *Listeria* spp.). Chlorine treatments include the use of electrolysed water as the active compound that remains in the process water is also free chlorine (FC). PAA, peroxyacetic acid. *Source*: EFSA outsourced activities (Gil et al., 2025).

Levels of total bacterial counts (TBC) in the process water were variable among the different scenarios (ranging from undetected up to 7.5 log_{10} CFU/100 mL) (Figure 7A). The highest TBC levels were observed when no water disinfection treatments were applied (scenarios ID 30, ID 31, ID 32 and ID 34). In scenarios where PAA was used (scenario ID 38), TBC ranged between 3 and 4 log_{10} CFU/100 mL. In those cases where FC was the active compound for water treatment (chlorine gas, sodium and calcium hypochlorite, and electrolysed water), TBC were variable, ranging from undetectable to 5 log_{10} CFU/100 mL. The lowest TBC (≤ 1 log_{10} CFU/100 mL) were mostly observed in those scenarios where chlorine-based disinfectants, including electrolysed water, were applied at FC residual concentration values ranging, in most of the cases, between 40 and 275 mg/L (Gil et al., 2025).

In Figure 7, electrolysed water has been included in the group of chlorine-based treatments as the active form that remains in the process water is FC. *E. coli* was only detected in those scenarios where no water disinfection treatment was applied (Figure 7B). A similar trend was observed for *Listeria* spp. counts when comparing the microbiological quality of process water when different strategies were applied. *Listeria* spp. was mostly detected in those scenarios where no water disinfection was applied. However, in scenario ID 37 (diced onions), where FC residual concentrations were in most cases very low (≤ 5 mg/L) and pH values above 7, *Listeria* spp. was detected between 1.2 and 2.2 log_{10} CFU/100 mL (Figure 7C). Detailed information related to the industrial data can be found in Gil et al. (2025).

The levels of VBNC bacterial cells in process water were determined in 4 scenarios of the fresh-cut FVH sector, all using chlorine-based water disinfection treatments (ID 37, ID 43, ID 44, ID 47). In all the cases, high levels of VBNC cells of TBC were observed. The levels of VBNC cells were about 2–3 $\log_{10}$ CFU/100 mL higher than that of the culturable, indicating the low efficacy of this intervention strategy reducing the induction of VBNC. Similar results were obtained also for the VBNC cells of TC, mostly in scenario ID 37. Due to the limited amount of data on VBNC bacterial cell levels, the results should be interpreted with caution. Given the data scarcity, it is not possible to draw conclusions comparing scenarios that use disinfectants and those that do not.

Based on the industrial data collected through EFSA's outsourced activities, the most relevant characteristics of the fresh-cut FVH sector are as follows:

(i) The temperature of the process water is usually controlled and cooled (6–11°C).
(ii) The amount of product that is processed during the operational cycle varies and mostly ranges between 700 and 3000 kg.
(iii) Intervention strategies are limited to the use of water disinfection treatments such as chlorine-based disinfectants, peroxyacetic acid (PAA) and electrolysed water. The most common water disinfection treatments are chlorine-based compounds such as sodium and calcium hypochlorite.
(iv) Water replenishment (or refreshment) is not applied as an intervention strategy. Only water refilling is used just to maintain the volume of the water tank or process lines.
(v) Water volumes in operation tanks vary widely (range: 800–3000 L). The most commonly used water source is municipal water, and rarely well water is used.
(vi) The product-to-water contact times during operations varies widely (range: 0.5–5 min), but in most of the cases, very short contact times were observed (e.g. less than 1 min).

3.2 | Identification of microbiological hazards and hazardous events linked to process water

In the Part 1 Opinion, it was concluded that *L. monocytogenes*, *Salmonella* and STEC were the most important hazards to be considered in fresh-cut FVH. This was based on an EFSA FoNAO opinion (EFSA BIOHAZ Panel, 2013), which reported European outbreaks between 2014 and 2020, and a literature review (publications published between 2010 and 15-2-2022) (EFSA BIOHAZ Panel, 2023). The prioritised hazard-product combinations are listed in Table 2.

TABLE 2 Prioritised microbiological hazard-product combinations in water used for post-harvest handling and processing operations for fresh-cut fruits, vegetables and herbs.

General fresh-cut FVH food category	Specific fresh-cut FVH food category	Microbiological hazards
Leaves	Leafy greens eaten raw as salads	*Listeria monocytogenes, Salmonella* spp., STEC, *Shigella* spp.,* norovirus, *Yersinia* spp., *Cryptosporidium* spp.
Leaves	Fresh Herbs	*Listeria monocytogenes, Salmonella* spp., STEC, *Shigella* spp.*
Fruits	NA	*Listeria monocytogenes, Salmonella* spp., STEC
Vegetable fruits	Peppers and aubergines	*Listeria monocytogenes, Salmonella* spp., STEC
Root and tuberous vegetables	Carrots	*Listeria monocytogenes, Salmonella* spp., STEC, *Yersinia* spp.

Abbreviations: FVH, fruit, vegetables and herbs; STEC, Shiga toxin-producing *Escherichia coli*.
*If imported raw product, NA = not available.

In EFSA's outsourced activities, analysis of *Salmonella* spp., *L. monocytogenes*, STEC (including O157:H7), norovirus and *Cryptosporidium* spp. was performed at six sampling time points in 19 different scenarios. All findings for pathogens are listed in Appendix C. *Salmonella* spp. and norovirus were the hazards most often detected in the process water of fresh-cut FVH. *Salmonella* spp. were detected several times in five different scenarios, namely in water used to wash shredded carrots and lettuce, endive, baby leaves and parsley. In two of these five scenarios, i.e. washing of endive and baby leaves, STEC was also detected on one sampling occasion (Appendix C). *L. monocytogenes* and *E. coli* O157:H7 were never detected in the process water of fresh-cut FVH. All the positive findings were from samples of non-disinfected water. In most of the cases, *Salmonella* spp. was found already at the first sampling time point, before any product had been washed and could further be detected throughout the whole operational cycle. However, it should be noted that all of these *Salmonella* spp. findings were confirmed by agglutination and not by PCR (Appendix C).

Norovirus and *Cryptosporidium* spp. were only analysed at a single sampling time point (i.e. at the last out of the 6 defined sampling time points) for each scenario. Whereas norovirus genomes were detected in several process water samples, *Cryptosporidium* spp. was never detected. Further, norovirus was the only pathogen detected in chlorine-disinfected water in the fresh-cut FVH scenarios. The detection method used for norovirus GI after adding PMAxx indicates the presence of intact virions but not their infectivity (Appendix C).

Based on the industrial data, including these pathogen findings, and the processing conditions described in Gil et al. (2025), different hazardous events can be expected. The most important identified hazardous events for the fresh-cut processing industry are:

a) **Incomplete removal of the water used in the water tank**. All scenarios with positive findings were from washing tanks filled with either well or municipal tap water. Still, *Salmonella* and STEC were found several times at the beginning of the operational cycle, even before any product had been washed (Appendix C), **which could indicate incomplete removal of water and/or poor cleaning and disinfection in-between operational runs**.
b) **Use of the same water to wash large volumes of product during the operational cycle without a well-managed intervention strategy**. Despite the relatively shorter operational cycles in the fresh-cut FVH sector, compared to fresh-whole and frozen FVH sectors, the shorter product water contact time leads to a **high product mass-to-water volume ratio**. Using the same water in a single handling and/or processing operation without applying a well-managed physical and/or chemical intervention strategy during the operational cycle will build up contamination in the water and may contribute to batch-to-batch contamination. In fact, **all positive findings of bacterial pathogens were from scenarios not deploying water disinfection** (Appendix C).
c) **Nutrient-rich water without replenishment**. Although the operational cycles are shorter in the fresh-cut sector, mostly 4–5 h with a max of 15 h (compared to other sectors such as fresh-whole and frozen sectors), the release of organic material quickly leads to a high chemical oxygen demand reducing the effect of disinfectants unless the water is replenished (or refreshed). This could be linked to the release of nutrients after the cutting operation.

3.3 | Analysis of microbiological and physico-chemical parameters of process water (data from EFSA's outsourced activities)

The data collected through EFSA's outsourced activities represents a unique and comprehensive database of industrial scenarios, offering valuable insights into the contamination dynamics of process water across various operations in the fresh-cut FVH sector (Gil et al., 2025). This database has been thoroughly analysed to explore potential relationships among the different parameters evaluated. The goal is to identify relationships among microbiological parameters as well as microbiological parameters and physico-chemical factors that can be used for the validation, verification and monitoring within the water management system procedures.

3.3.1 | Relationship among microbiological parameters

In order to understand the relationship between the levels of potential microbial indicators and the detection of pathogens in process water of fresh-cut FVH, the microbiological data was analysed in three steps:

3.3.1.1 | *Exploratory analysis of the results by individual samples*

Bacterial pathogens were detected in 50 out of 456 water samples analysed, belonging to 5 out of 19 scenarios. *Salmonella* spp. was the most frequently detected pathogen (agglutination-confirmed), while PCR-confirmed STEC was found in two samples. *L. monocytogenes* and *E. coli* O157:H7 were not detected in any sample. Given these results, the data associated with the detection of *Salmonella* spp. together with STEC (as enteric pathogens) were used to assess the suitability of TBC, TC, *E. coli* and *Listeria* spp., as potential microbial indicators of pathogen contamination.

In Figure 8, the counts of TBC, TC, *E. coli* and *Listeria* spp. as $\log_{10}$ CFU/100 mL for individual samples are displayed as a combined boxplot for samples in which the above-mentioned enteric pathogens (agglutination-confirmed *Salmonella* and/or PCR-confirmed STEC)[9] were either detected or not detected.

[9] 48 out of 50 detected enteric pathogens were *Salmonella* spp.-positive samples, with all 48 agglutination-confirmed.

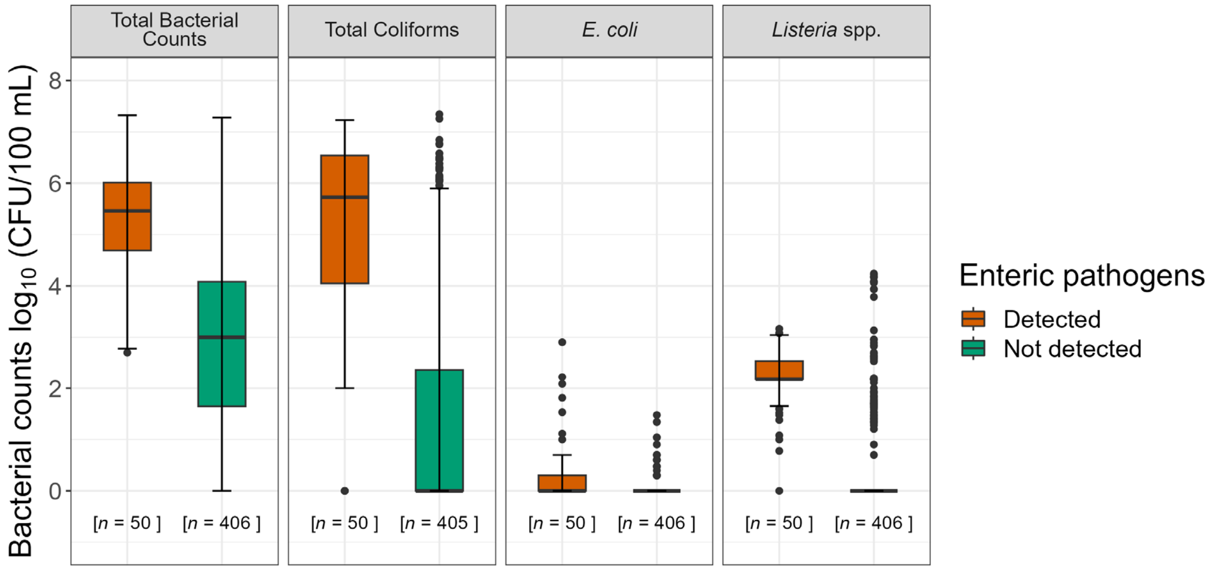

FIGURE 8 Box plot of levels ($\log_{10}$ CFU/100 mL) of total bacterial counts (TBC), total coliforms (TC), *E. coli* and *Listeria* spp. in individual samples of process water taken from different processing operations of fresh-cut FVH scenarios in which the enteric pathogens (agglutination-confirmed *Salmonella* and/or PCR-confirmed STEC)[10] were detected (orange) and not detected (green). The numbers in square brackets [*n* =] represent the number of process water samples in each box plot. The results of the microbial indicators below the respective LOD were set to 0 $\log_{10}$ CFU/100 mL. *Source*: EFSA outsourced activities (Gil et al., 2025).

The descriptive analysis of the empirical distribution of the data considering each individual sample, without accounting for the association among the sampling observations due to the multilevel hierarchical structure of the data, was performed first. The main results are described below.

The levels of **TBC** in samples positive for enteric pathogens (agglutination-confirmed *Salmonella* or PCR-confirmed STEC)[11] (with a mean of 5.25 $\log_{10}$ CFU/100 mL) tend to be generally higher than the levels in samples negative for enteric pathogens (with a mean of 2.89 $\log_{10}$ CFU/100 mL). More specifically:

- TBC were above 10^6 CFU/100 mL in 26% of the samples positive for enteric pathogens and in 8% of the samples negative for enteric pathogens.
- TBC were above 10^5 CFU/100 mL in 64% of the samples positive for enteric pathogens and in 13% of the samples negative for enteric pathogens.
- TBC were above 10^4 CFU/100 mL in 84% of the samples positive for enteric pathogens and in 27% of the samples negative for enteric pathogens.

The levels of **TC** found in samples positive for enteric pathogens (with a mean of 4.84 $\log_{10}$ CFU/100 mL) were generally higher than in samples negative for enteric pathogens (with a mean of 1.54 $\log_{10}$ CFU/100 mL).

More specifically:

- TC were above 10^4 CFU/100 mL in 76% of the samples positive for enteric pathogens and in 15% of the samples negative for enteric pathogens.
- TC were above 10^3 CFU/100 mL in 83% of the samples positive for enteric pathogens and in 19% of the samples negative for enteric pathogens.
- TC were above 10^2 CFU/100 mL in 90% of the samples positive for enteric pathogens and in 32% of the samples negative for enteric pathogens.

The levels of ***E. coli*** in process water were below 100 CFU/100 mL in almost all the samples taken in the fresh-cut FVH scenarios. Samples positive for enteric pathogens (agglutination-confirmed *Salmonella* or PCR-confirmed STEC) showed slightly higher levels of *E. coli* than samples in which these enteric pathogens were not detected. For instance, *E. coli* was below the LOD (1 CFU/100 mL) and below 10 CFU/100 mL in 92% and 99%, respectively, in the samples negative for these pathogens and 63% and 88% of the samples positive for these pathogens.

Listeria spp. was also detected at higher levels in samples for which enteric pathogens were detected compared with samples negative for these pathogens. In fact, *Listeria* spp. could be enumerated (> 1 CFU/100 mL) in 100% of the samples positive for enteric pathogens (with 88% of samples showing levels ≥ 100 CFU/100 mL), while this potential microbial

[10]48 out of 50 detected enteric pathogens were *Salmonella* spp.-positive samples, with all 48 agglutination-confirmed.

[11]48 out of 50 detected enteric pathogens were *Salmonella* spp.-positive samples, with all 48 agglutination-confirmed.

indicator group could be enumerated only in 24% of samples negative for enteric pathogens (with levels ≥ 100 CFU/100 mL being observed in 15% of the samples).

3.3.1.2 | *Analysis of pathogen detection within the operational cycle (by sampling visit)*

The data were also analysed to detect pathogens within each sampling visit (lasting for the duration of the processing operation) of each scenario. Within the total 38 different visits, agglutination-confirmed *Salmonella* spp. was detected in at least one sample of 9 sampling visits, which corresponded to the following scenarios: ID 30 (in the two visits), ID 31 (in the two visits), ID 32 (in one visit), ID 33 (in the two visits), ID 34 (in the two visits), while confirmed STEC was detected in two samples belonging to different scenarios (ID 31 and ID 32). In scenario ID 31 and ID 32, both pathogens were detected at different sampling time points along the operational cycle (Appendix C).

The relationship between the occurrence of indicator microorganisms above a given threshold and the detection of enteric pathogens (agglutination confirmed *Salmonella* spp. and/or confirmed STEC)[12] within each sampling visit was assessed. For this purpose, the OR,[13] its 95% CI and the statistical significance were calculated for three different thresholds for TBC, TC, *E. coli* and *Listeria* spp. as potential microbial indicators (Appendix D). According to the results, when TBC were above 10^5 CFU/100 mL, TC above 10^4 CFU/100 mL, or *E. coli* or *Listeria* spp. were detected (i.e. counted above 1 CFU/100 mL), the odds of detecting enteric pathogens[14] were more than 100-fold (95% CI: ranging from around 9.0–around 1400, $p < 0.001$) the odds of detecting enteric pathogens in observations with microbial indicators levels below the respective thresholds. Therefore, both the analysis of the results for individual samples (i.e. box plots above) and through the OR grouped by sampling visits resulted in similar conclusions for the potential microbial indicator for the process water of fresh-cut FVH. It should be noted that the odds are estimated with high sampling uncertainty as shown by the large 95% CI.

3.3.1.3 | *Analysis by multivariable logistic mixed effect modelling (hierarchical structure)*

A multivariable logistic mixed effect model was developed to explain the potential effect on the detection of the microbial pathogens of the microbial indicator levels considering data from all the FVH sectors and, only for *L. monocytogenes*, also considering the effect of the analytical methods applied to confirm the detection (carbohydrate fermentation testing vs. PCR). The hierarchical structure of the data was accounted for using random effects (more details about the complete model are available in Annex B).

For this sector, this logistic model considered only cases of *Salmonella* spp. detection, whereas previous analyses (boxplots and OR) merged the detection of *Salmonella* spp. and STEC as enteric pathogens.[15] This difference is considered negligible, seen in very few cases of STEC detection. Annex B provides a detailed description of the modelling and its outcomes.

In summary, Model 1 predicted that the odds of detecting *Salmonella* spp. in process water of fresh-cut FVH would decrease by a factor of 0.58 (95% CI 0.37–0.91) for each Log_{10}-unit increase of TBC. Model 2, on the other hand, did not highlight any significant effect on the odds of detecting *Salmonella* in process water of fresh-cut FVH for any potential microbial indicator.

The two models did not indicate any effects of the increase of the potential microbial indicator levels on the odds of detection of *Salmonella*, except for the case of TBC, which is difficult to interpret. This is probably due to a series of uncertainties affecting the data. First of all, the parameter estimates were not robust (i.e. they varied depending on the variables included), which suggests a degree of redundancy in the ability of the levels of microbial indicators to explain the odds of *Salmonella* detection.

In addition, the two models show a singularity issue, suggesting that collinearity could still affect the results and make the estimate of some random effects unreliable. However, dropping one of the random effects is not advisable, considering the hierarchical structure of the data. Therefore, the issue could not be resolved.

The only consistent conclusion among both models about the relationship between the potential microbial indicators and the detection of pathogens in process water is that random effects (i.e. 'scenario', 'sampling visit' and 'sampling time points') play an important role. Particularly, the parameter 'scenario' is associated with the largest variability among the random effects when predicting the detection of *Salmonella*.

It is worth noting that this 'scenario' parameter combines several operational features, such as the specific combination of FVH product, type of operation and operational conditions, each including additional specific characteristics. The individual effect of each of these features and characteristics could not be investigated through modelling and remains an open issue.

Therefore, the suitability of the potential microbial indicators for verification purposes within the water management system should be validated under the specific operational conditions of each FBOp, as described in Section 3.5.

[12] 48 out of 50 detected enteric pathogens were *Salmonella* spp.-positive samples, with all 48 agglutination-confirmed.

[13] Odds measured how likely the detection of the pathogen is, and is calculated when the indicator is below and above the given threshold.

[14] 48 out of 50 detected enteric pathogens were *Salmonella* spp.-positive samples, with all 48 agglutination-confirmed.

[15] 48 out of 50 detected enteric pathogens were *Salmonella* spp.-positive samples, with all 48 agglutination-confirmed.

3.3.2 | Relationship between microbiological and physico-chemical parameters

Despite comprehensive statistical analysis, including techniques such as PCA, the data set for fresh-whole FVH did not reveal any critical parameters suitable for use in operational monitoring. Statistical data analysis tools, such as PCA and others, rely on variation within a data set to identify meaningful relationships between factors and data sets lacking variation hinder the ability to draw strong conclusions. The studies performed under real industrial settings conditions (as the ones observed in the scenarios sampled in EFSA's outsourced activities) often suffer from this limitation because the actual practices applied in commercial processing and handling conditions minimise variation in most physico-chemical parameters. On several occasions, the microbial loads fall below detectable levels or are randomly distributed.

Although the PCA approach could not unravel statistical correlation between physico-chemical and microbiological parameters, it is worth mentioning that TC and *Listeria* spp. were present in samples with a residual FC concentration below 100 mg/L under chlorine-based disinfection or in the presence of electrolysed water, irrespective of the water source (municipal tap water, surface water, or well water).

To ensure real-time monitoring of process water quality, identifying critical physico-chemical parameters that correlate with the microbiological quality of the process water is essential. It would allow the online monitoring of physico-chemical parameters as a proxy for microbiological quality.

3.4 | Preventive measures: Good hygiene and good manufacturing practices in water management, distribution and storage systems

All 19 scenarios were systematically screened, and hazardous events were identified (Section 3.2) and linked to specific preventive measures. Below, in Table 3, specific highlights for the fresh-cut FVH production are formulated as pre-start measures (i.e. to set up the water management system), restart measures (i.e. to start up again after technical interference or temporary work) and routine measures (i.e. in the daily routine operation of an industry). A full description of generic pre-requisite measures (13 PRPs) can be found in the EC Commission Notice 2022/C 355/01[16] and PRPs specific for water management, distribution and storage systems in the Part 1 Opinion (EFSA BIOHAZ Panel, 2023).

[16]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, pp. 1–58.

TABLE 3 Specific points of attention in the preventive measures, as defined in the EC Commission Notice 2022/C 355/01[a] and Part 1 Opinion (EFSA BIOHAZ Panel, 2023), related to Good Hygiene and Good Manufacturing Practices in water management, distribution and storage systems for post-harvest water use in handling and/or processing operations of fresh-cut FVH, based on the identified hazardous events (Section 3.2).

Preventive measure	Specific attention to preventive measures for fresh-cut FVH		
PRE-REQUISITE (EC Commission Notice 2022/C 355/01)	**PRESTART MEASURES**	**RESTART MEASURES**	**ROUTINE MEASURES**
PRP 1: Infrastructure and fit-for-purpose building and equipment	Hygienic design and special expertise are needed to set up the infrastructure of the water management system to avoid problems and reduce routine inspection/maintenance Set up a well-designed and managed water disinfection system as an intervention measure (installation of pumps, dosing systems, sensors, valves, etc.)	Every time the processing line is restarted, the infrastructure and equipment should be revised	A monthly visual check based on a checklist of infrastructure (hygiene and condition) Monitoring the proper working of the water management system (see Section 3.6)
PRP 2: Cleaning and disinfection	The equipment used for the cleaning and disinfection of the water distribution and storage systems needs to be revised before activities are started	In case of restart after a prolonged stand-still (as in the case after technical maintenance), cleaning and disinfection need to be conducted to prevent microbial contamination of water and avoid biofilm formation	Periodically performing cleaning and disinfection procedures, including deep cleaning and disinfection procedures. Activities might include effective soil removal, biofilm prevention and removal, and pathogen inactivation Dedicated time surely needs to be foreseen when continuous and/or long runs occur during fresh-cut FVH processing – Spot visual checks – Daily visual checks Perform microbiological environmental testing
PRP 4: Technical maintenance and calibration	Proper equipment installation is needed to prevent contamination of fit-for-purpose water with potentially contaminated water (such as between potable water fill lines and early stages of washing where fit-for-purpose water is used), including the water disinfection system	Proper re-installation of equipment is needed (e.g. connecting pipelines) to prevent contamination of fit-for-purpose water with potentially contaminated water, surely, after a prolonged period of not using the water management system, e.g. due to technical maintenance	Routine inspection and maintenance of equipment are needed to prevent contamination of fit-for-purpose water with potentially contaminated water Monitoring programmes of technical maintenance may consist of (a) inspections of records of the functionality and maintenance status of equipment and (b) inspections of the abstraction area and the treatment, storage and distribution infrastructure without prejudice to monitoring requirements Monitoring equipment needs to be calibrated, e.g. thermometers, water flow meters and disinfection dosing systems, so that proper use can be guaranteed In case a deviation is found during the routine inspections and/or cleaning activities, a replacement of water distribution systems needs to be foreseen to avoid contamination due to biofilms, e.g. filters, tubing, fittings to connect tubes or nozzles to make small water droplets in hydrocooling installations

TABLE 3 (Continued)

Preventive measure	Specific attention to preventive measures for fresh-cut FVH		
PRE-REQUISITE (EC Commission Notice 2022/C 355/01)	**PRESTART MEASURES**	**RESTART MEASURES**	**ROUTINE MEASURES**
PRP 8: Water and air control	Cooling post-harvest water to reduce microbial growth	Cooling post-harvest water to reduce microbial growth	Cooling post-harvest water to reduce microbial growth The microbiological quality of ice should also be considered to avoid potential contamination (in case ice is used to cool products)
	The frequency of complete removal of water in tanks/process lines needs to be established	Every time the processing line is restarted, the water in the processing lines/tanks should be completely removed and new fresh water inserted	Routine inspection of the frequency of complete removal of the water in the tanks/processing lines is needed to avoid prolonged use of the processing water (and, therefore, avoid the use of the same water to wash large volumes of product during the operational cycle)
PRP 9: Personnel (hygiene, health status)	Appropriated training for operators and team leaders is required due to the nature of manual labour (e.g. application of acid to control pH, manual sorting) and to create a positive food safety culture and awareness of hygiene and microbiological food safety		Retake the training with a certain frequency to create awareness among personnel on microbiological hygiene and the safety of water and produce
PRP 12: Working methodology	Personnel following work descriptions and standard operating procedures (SOP)	Personnel following work descriptions and standard operating procedures (SOP)	Personnel following work descriptions and standard operating procedures (SOP) **SOP includes logistic management:** e.g. optical dirty after optical clean products, or if different product categories are processed: covered (e.g. peas) before above ground (open e.g. green leaves, tomatoes, peppers) before root vegetables (e.g. carrots) **SOP includes reusing water:** use the process water applied for 'cleaner' processing steps as input water for more dirty steps, e.g. last washing/rinsing water to be reused for the first washing of FVH **SOP includes the use of disinfection techniques:** clear explanation of the dose, monitoring, etc., and what to do in case the disinfection is not working appropriated (e.g. actions to take to process and product)

Abbreviation: FVH, fruit, vegetables and herbs.

[a]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, pp. 1–58.

3.5 | Intervention measures: Water disinfection and/or water replenishment

Intervention measures included steps in the post-harvest handling and processing operations aiming to avoid the microbiological contamination of the process water, thus preventing the accumulation of microorganisms in the water and the consequent cross-contamination of the processed fresh-cut FVH. In the Part 1 opinion (EFSA BIOHAZ Panel, 2023), two main intervention measures were identified: water replenishment and water disinfection treatments. However, based on EFSA's outsourced activities, only chemical water disinfection treatments were applied by the FBOps from the fresh-cut FVH sector (Gil et al., 2025) (Table 4). In this case, four Scenario Groups (SG) were identified, as presented in Table 5, which cover the above-mentioned intervention measures. It is critical to note that the application of an intervention measure needs to be tailored to the product and processing line, and it should be integrated into the water management strategy, which includes validation, operational monitoring and verification to demonstrate its performance. Therefore, the information provided in this section should be considered as illustrative.

TABLE 4 Types of intervention measures considered in this scientific opinion and the number of scenarios found in the fresh-cut FVH industrial data included in EFSA's outsourced activities ($n = 19$ scenarios from three different European countries) (*Source*: EFSA outsourced activities, Gil et al., 2025).

Intervention measures	Number of scenarios from EFSA's outsourced activities
None	6
Water Disinfection (WD)	13
Water Replenishment (WR)	0
Water Disinfection and Water replenishment (WD + WR)	0

To illustrate the different SG identified within the industrial cases included in the EFSA outsourced activities and also other situations that were not found among the industrial cases, representing adequate water safety management (e.g. SG 2.2 and SG 4) (Table 5), a mathematical model was used to simulate hypothetical scenarios representative of the fresh-cut FVH sector (Section 2.2.2). The mathematical model has been implemented in a public user-friendly tool (https://r4eu.efsa.europa.eu/app/WaterManage4You).

To compare the outputs of the model when different intervention measures are applied and with the SG 1 (no intervention), all the simulations refer to common hypothetical processing conditions of a fresh-cut product (i.e. leafy greens), based on scenario ID 43, sampling visit 2 (Gil et al., 2025), with an operational cycle of 6.5 h, running under the same operational conditions (e.g. volume of water 2300 L and total amount of product 5624 kg) as shown in Figures 9 and 10 and Table 5. In this case, a continuous product load was assumed during the operational cycle. A constant addition of the product facilitates the water management, as it has been proven that irregular peaks of product addition during the operational cycle, which is characteristic of some sectors (fresh-whole FVH), challenges the management of the microbiological quality of process water (EFSA BIOHAZ Panel, 2025b – Part 3 Opinion).

Tables E1 and E2 in Appendix E summarise all the model parameters and input data used for variables in the model simulations of all the SG for the fresh-cut FVH sector.

TABLE 5 Conditions[a] of application of the simulated intervention measures and physico-chemical parameters (FC, pH and temperature) used as input values for model simulations of all the scenario groups representative of the fresh-cut FVH sector.

	Intervention measure conditions			Physico-chemical parameters	
Scenario group	**Initial chlorine concentration (mg/L)**	**Mode and dose of chlorine addition**	**Water addition (L/min)**	**pH**	***T* (°C)**
Scenario Group 1	0	None	Water refilling: 0.5	Not adjusted: 7.1–8.4	Not adjusted: 5–8
Scenario Group 2.1	20	Discrete: 10,000 mg every 15 min	Water refilling: 0.5	Not adjusted: 7.1–8.4	Not adjusted: 5–8
Scenario Group 2.2	20	Continuous: 1000 mg/min	Water refilling: 0.5	Adjusted: 6	Not adjusted: 5–8
Scenario Group 3	0	None	Water replenishment: 25	Not adjusted: 7.1–8.4	Not adjusted: 5–8
Scenario Group 4	20	Continuous: 1000 mg/min	Water replenishment: 25	Adjusted: 6	Not adjusted: 5–8

Abbreviations: FC, free chlorine; FVH, fruit, vegetables and herbs.

[a]Based on scenario ID 43, sampling visit 2 from EFSA's outsourced activities (Gil et al., 2025), with an operational cycle of 6.5 h, running under the same operational conditions (e.g. volume of water 2300 L and total amount of product 5624 kg, contact time 1 min).

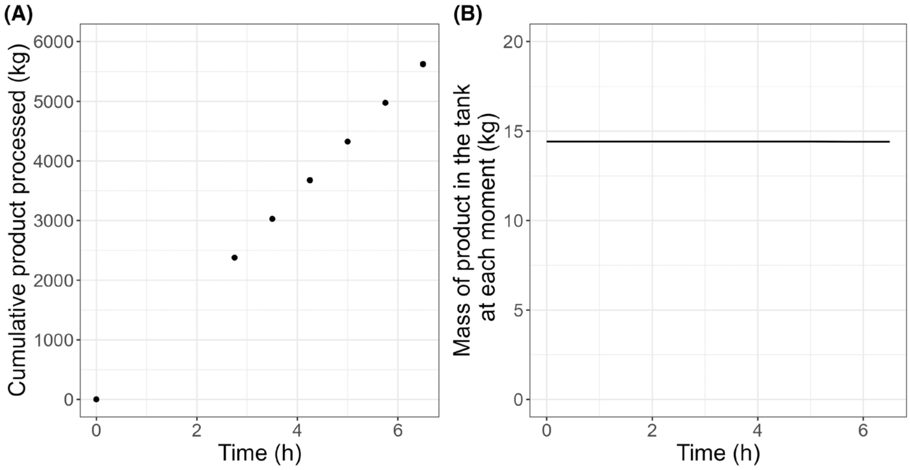

FIGURE 9 Cumulative mass of the product being processed (A) and the mass of the product in the tank at each moment (B) during the 6.5-h operational cycle used in the simulations of the scenario groups selected as representative of the fresh-cut FVH sector.

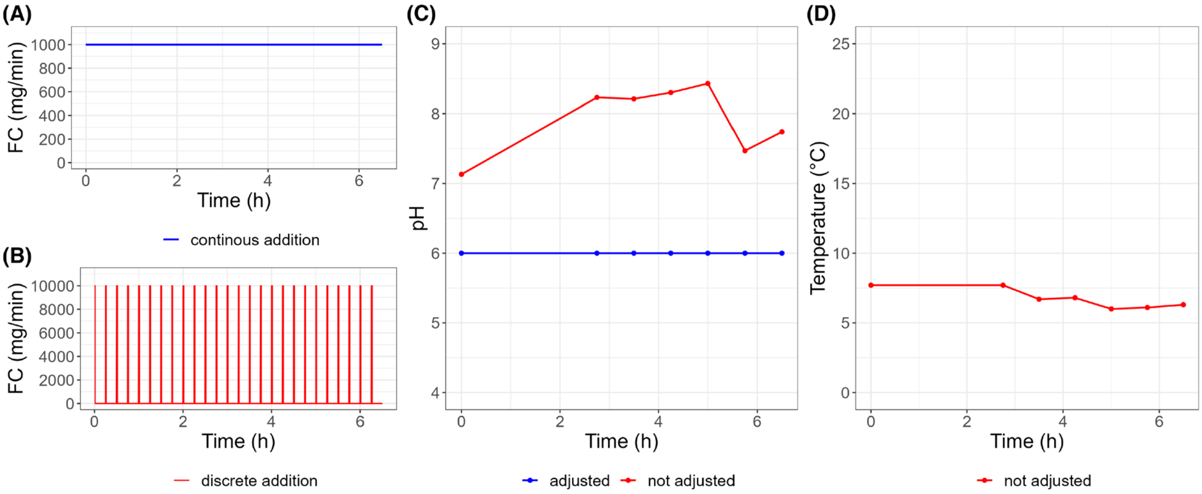

FIGURE 10 Changes in physico-chemical parameters free chlorine (FC) dosing (A: continuous addition, B: discrete addition), pH (C) and temperature (D) used as model inputs for the simulation of the scenario group of the fresh-cut FVH sector, corresponding to a discrete application of water disinfection treatments and no adjustment of the physico-chemical characteristics of the water (SG 2.1), and the scenario groups associated with a continuous application of water disinfection treatments and adjustment of the physico-chemical characteristics of the water (SG 2.2 and SG 4).

Figure 11 shows the simulations provided by the mathematical model for different parameters, including FC and hypochlorous acid (HOCl) (A), accumulation of COD (B) and levels of Total Bacterial Counts (C) based on the different defined scenario groups (SGs 1, 2.1, 2.2, 3 and 4). Each column of Figure 11 refers to one type of SG.

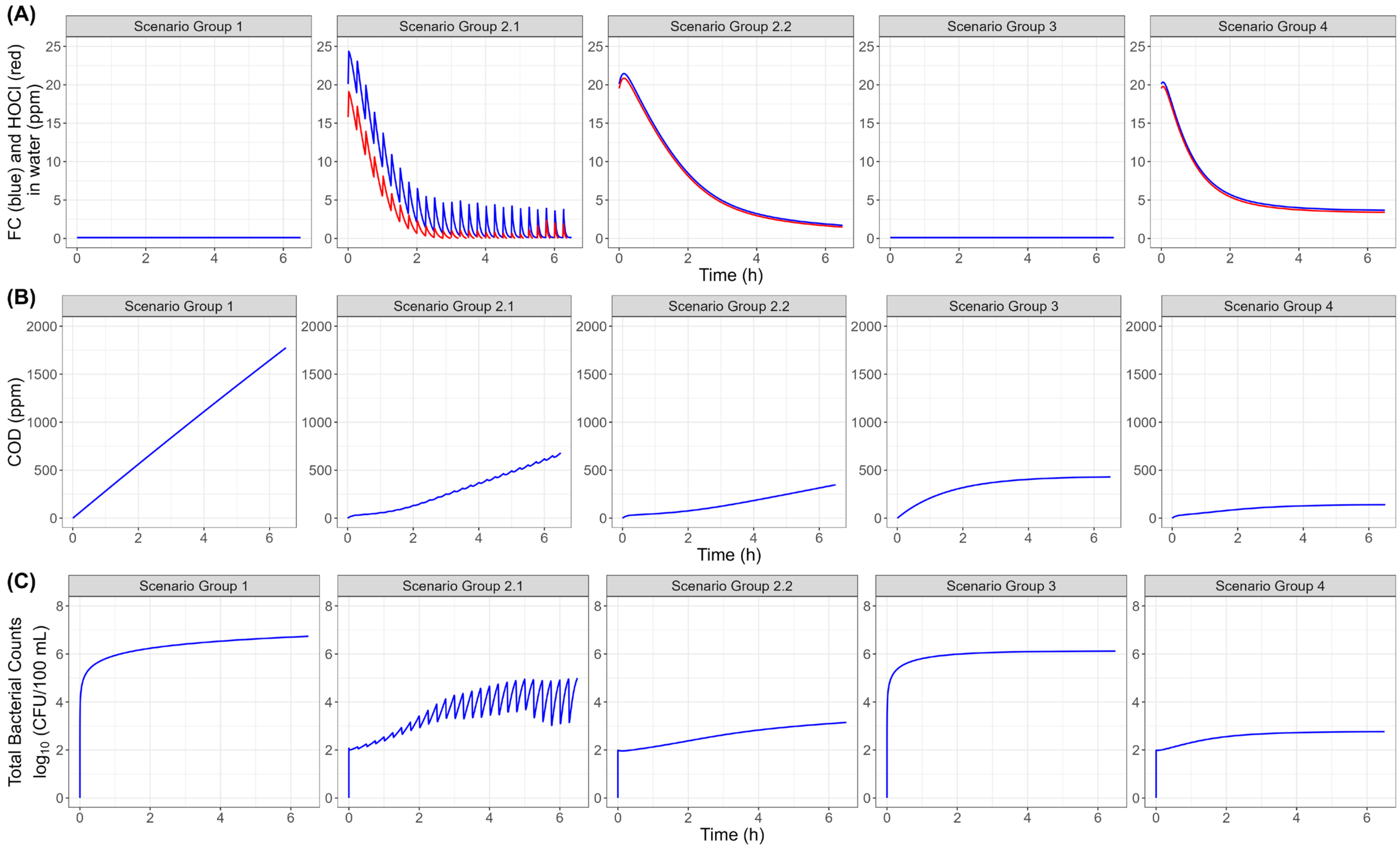

FIGURE 11 Model simulation outputs including free chlorine (FC) and hypochlorous acid (HOCl) (A), accumulation of the chemical oxygen demand (COD) (ppm correspond to mg/L) (B) and Total Bacterial Counts (C) for the different scenario groups in the fresh-cut FVH sector (see Table 5 for the main characteristics of the scenario groups).

Scenario Group 1: The simulations included in this group represent a situation commonly observed within the industrial data collected through EFSA's outsourced activities (Gil et al., 2025), where no intervention strategies were applied (i.e. no water disinfection and no water replenishment). In this SG, no FC is detected (Figure 11A), and only water refilling at a constant rate (0.5 L/min) is considered (Table 5). The model simulations clearly show that in the absence of any intervention strategy, the COD (Figure 11B) and the microbial load (Figure 11C) of the initially clean water constantly increase throughout the operational cycle, reaching levels up to 1800 mg/L and ca. 6–7 log_{10} CFU/100 mL, respectively (Figure.11). Under these conditions, the water management system is not able to control the accumulation of bacteria in the process water.

Scenario Group 2 resembles a handling and processing operation with chlorine-based disinfection treatment of process water without water replenishment, but only water refilling at a constant rate (0.5 L/min) (Table 5). Two different sub-scenario groups were addressed:

- **Sub-scenario group 2.1** represents a scenario in which the physico-chemical parameters indicated that the water management system was not adjusted to achieve the fit-for-purpose microbiological quality. The water disinfection treatment was applied without properly adjusting the chlorine dosing regimen or the physico-chemical parameters. In this scenario, the quantity of disinfectant added during the operational cycle was too low and was applied discretely; the pH was not optimal, and the temperature was not adjusted. Under such conditions, the concentration of FC in water and hypochlorous acid is minimal during the operational cycle (Figure 11A). As a result, COD rises up to 800 mg/L (Figure 11B) and microbial load increases close to 5 log_{10} CFU/100 mL (Figure 11C).
- **Sub-scenario Group 2.2** shows a scenario where disinfectant was continuously added during the operational cycle, and the physico-chemical parameters of the water (pH and temperature) were adjusted to values favouring the action of the FC. Under these conditions, a residual concentration of FC (and hypochlorous acid) was present during the operational cycle. However, the decrease from the initial concentration (Figure 11A) and the increase in COD concentrations were reduced to levels below 500 mg/L (Figure 11B). Compared with the above-described scenarios, the accumulation of microorganisms was controlled between 2 and 3 log_{10} CFU/100 mL (Figure 11C). The presence of a residual concentration of hypochlorous acid, i.e. the fraction of FC with the highest antimicrobial efficacy, above 5 mg/L at the beginning of the operational cycle results in a low accumulation of microorganisms in the process water (< 3 log_{10} CFU/100 mL) throughout almost all the operational cycle. Although the same flow rate of chlorine-dosing was kept during the operational cycle, the chlorine demand increased during the operational cycle due to the increase of organic matter with the addition of product being processed (as shown by COD), and it is reflected by a reduction of the residual concentrations of FC and hypochlorous acid below 5 mg/L (at about 3 h of the operational cycle). The microbial load increased slightly above 3 log_{10} CFU/100 mL when approaching the last 25% of the operational cycle (about 6 h).

Scenario Group 3 refers to a handling or processing operation with water replenishment as the only intervention, without the addition of any water disinfection treatment (Figure 11A). The water replenishment is defined in the model by increasing the addition of water from 0.5 L/min (just water refilling) to 25 L/min (Table 5). A water flow rate of 25 L/min was selected as it was considered a flow rate that can reasonably be applied by the industry. Although a higher flow rate than 25 L/min may be possible, this option was not considered in this study. The COD of the process water is kept at very low concentrations when compared to SG 1 and 2, mostly due to the dilution effect of the high addition of water (Figure 11B). According to the model simulations, the application of such an intervention alone is much less efficacious than water disinfection treatments in controlling the accumulation of microorganisms in the water, mostly because the concentration of microorganisms accumulate in a logarithmic scale and, thus, much higher water addition rate would be needed to observe a dilution effect (Figure 11C).

Scenario Group 4 represents the situation where water disinfection and replenishment (25 L/min) interventions are applied as intervention measures. In this case, the simulation includes the same conditions applied in SG 2.2, adjusting both chlorine dosing to a constant addition and the physico-chemical parameters that favour the antimicrobial activity of FC (Figure 10). The water replenishment rate used in this SG is the same as in SG 3 (Table 5). Again, a water flow rate of 25 L/min was selected as it was considered a flow rate that can reasonably be applied by the industry. Although a higher flow rate than 25 L/min may be possible, this option was not considered in this study. Based on the output of the model, it is observed that the dilution effect provided by water replenishment, represented by the addition of large volumes of water (25 L/min), has an impact on avoiding the accumulation of organic matter in the process water; thus, COD remains below 250 mg/L (Figure 11B).

As a consequence, compared with SG 2.2, since the oxidation of FC by organic matter is lower, despite the initial higher dilution of the FC residual concentration, there is a rapid depletion to 5 mg/L after 2 h and maintenance close to 5 mg/L until the end of the operational cycle (Figure 11A). Therefore, the combination of water disinfection (to control the accumulation of microbial load) and water replenishment (to dilute organic determining the FC demand) improves the water management of the system as the microbial load of the process water is well maintained showing levels close to 3 log_{10} CFU/100 mL with residual FC keeps slightly below 5 mg/L (Figure 11C).

3.6 | Validation of intervention measures

For the present opinion, the aim and scope (Step 1 in Figure 11, see EFSA BIOHAZ Panel, 2023) of the validation is to demonstrate that a specific processing and/or handling operation for a specific fresh-cut FVH, working under reasonably foreseeable operating conditions ensures that the microbiological quality of the process water is maintained and, thus, the cross-contamination of the fresh-cut FVH by the process water is minimised. Other validation purposes mentioned in Opinion Part 1 are out of the scope of this opinion (such as mapping the washing equipment to identify the worst-case locations showing the lowest concentration/dose of disinfectant, where the sensors for key monitoring parameters should be placed). The validation allows the definition of the appropriate operational monitoring criteria associated with water management strategies, which will be based on critical limits of certain physico-chemical parameters of the process water. The validation also allows the selection of the most appropriate microbial indicators and their thresholds to be used to verify the quality of the process water.

The subsequent steps of the validation (Steps 2 until 5, Figure 11, see EFSA BIOHAZ Panel, 2023) for this sector include:

(A) Identification of the handling and processing operation where water is applied in the fresh-cut FVH production

For the specific processing operations applied in fresh-cut FVH, the typical characteristics of the water used need to be set, based on the flow chart in Figure 1 defined for an FBOp where water is applied in the production steps, e.g.:

- Pre-washing, Washing and Rinsing: recirculation of water; refill to compensate for the loss of water and/or use of replenishment
- Transport water – water used in pre-sorting and cutting of FVH (not applied for all commodities)

(B) Identification of the fixed and variable process and product-related factors

For each of the specific processing operations applied in fresh-cut FVH, the following operation conditions of the process/es need to be defined to account for the variability of the actual operating conditions of each process line, to be able to test the robustness of the water management strategies:

(1) Fixed process parameters (remain constant in every processed batch), e.g. equipment dimensions, water source, water filtration and type of intervention measure (if applicable).
(2) Product-related factors, both

- Fixed factors that remain constant in every processed batch, such as type(s) of commodity, product feed rate, and
- Those that may vary considerably from batch to batch include the amount of dust/soil/organic matter. This last factor is particularly important as it may show a range of variability considering the climatic conditions at the moment of harvesting the FVH, e.g. heavy rainfall, as more soil can be present on the FVH and thus in the water.

(3) Water-related factors, including

- Fixed factors that remain constant in every processed batch, e.g. water hardness, water temperature, water flow, refreshment or replenishment rate (volume of water replaced in a certain time frame) and the product-to-water ratio.
- Dynamic factors vary along the processing cycle, such as relevant physico-chemical parameters, including pH, organic matter measured as COD and UV absorbance, and initial microbial load of the water at the start of the operation (time 0) in relation to the microbial load in the process water.

(4) Water disinfection treatment-related factors (if applicable), including

- Fixed factors remain constant in every processed batch, e.g. type of disinfectant (commercial product and its composition), concentration of application, mode of application (discrete or continuous) and disinfectant dose.
- Dynamic factors vary along the processing cycle, e.g. the level of disinfectant and the dosing regimen, as well as the temporal and spatial changes of the residual concentration of the disinfectant (in the active form).

Examples of industry data on these factors and their combinations can be retrieved from EFSA's outsourced activities (Gil et al., 2025). However, these activities were not designed to carry out a validation study; therefore, no scenario could be used as a reference for the validation process.

For a preliminary in-silico study of the validation assay, a decision support tool based on mathematical models can be used to evaluate whether the foreseen conditions of the water treatment (e.g. replenishment rate, concentration of disinfectant, adjustment of pH) could be suitable to maintain the microbiological quality of the process water. Section 3.5 of this opinion illustrates the impact of certain conditions in applying intervention or their combinations on the resulting process water quality using the mathematical model described in the Part 2 Opinion (EFSA BIOHAZ Panel, 2025a). The mathematical model has been implemented in a public user-friendly tool (https://r4eu.efsa.europa.eu/app/WaterManage4You).

An FBOp can introduce the identified fixed and variable product and process-related factors as inputs of the tool to assess the impact of foreseeable ranges of variation of these parameters (e.g. range in pH values, range in product mass, range in temperature) on the resulting microbiological quality and its accumulation during an operational cycle. Based on the outcomes of such simulations, the performance standards to be validated can be set or re-adjusted to achieve the desirable water quality according to the model. The use of the model narrows down the scope of the experimental study in the production plant (see subsections C and D below). However, the currently available models are limited to the behaviour of total bacterial counts as microbial indicators and chlorine-based disinfectant in combination with or without water replenishment as intervention measures.

(C) Selection of indicator parameters and their performance standards

In the validation study, all relevant parameters related to factors 1 to 4 (indicated above) should be collected for each step of the production where water is involved. In combination with the actual measurement of the physico-chemical parameters and microbiological analysis of the sampled water, it must demonstrate that performance standards of the microbiological load are maintained.

As no *L. monocytogenes* was detected at all in the fresh-cut sector data set, no relationship between potential microbial indicator groups and this pathogen could be established (see Section 3.3.1).

Although EFSA's outsourced activities were not aimed at performing a validation study, in agreement with the literature (Part 1 opinion), the collected data suggest that it is possible to use TBC, TC, *E. coli*, *Listeria* spp. as potential indicators for the detection of enteric pathogens in the process water for fresh-cut FVH. For TBC above 10^5 CFU/100 mL, TC above 10^4 CFU/100 mL, or detection of *E. coli* or *Listeria* spp. (i.e. counts above 1 CFU/100 mL), the odds of detecting enteric pathogens were about 100-fold higher than the odds of detecting enteric pathogens when these microbial indicators were below these thresholds.

Some recommendations on the microbiological quality of agricultural water used in post-harvest handling and processing operations are already available. For instance, the EU Commission Notice (2017/C 163/01) on guidance document on addressing microbiological risks in fresh fruits and vegetables (fFVs) at primary production (including the associated operations of washing/rinsing, sorting, transport, cooling) through good hygiene, indicates *E. coli* thresholds depending on the intended use of the water, the water source, the characteristic and the nature of the fFVs. The Commission Notice also provides support tools to evaluate the required microbiological quality of the agricultural water (Annex II and III of EU Commission Notice (2017/C 163/01)).

Despite the fact that all the above mentioned indicators and levels could be regarded as possible performance standards to control the detection of enteric pathogens in process water, outputs from EFSA's outsourced activities (Gil et al., 2025) and the scientific literature (Part 1 opinion) also showed that many features specifically associated with each FVH product, type of processing operations, operational conditions, etc., determine the actual suitability of a bacterial group and its threshold to indicate the possible detection of a pathogen in the process water. Therefore, selecting a specific indicator and its threshold should be done and justified by the FBOps based on the results of its specific validation study. Moreover, setting the thresholds should also be based on the fit-for-purpose water concept depending on the specific handling and processing operation and the intended use of the FVH (e.g. RTE or non-RTE FVH, respectively) among others (FAO/WHO, 2021).

In this sector-specific opinion for the fresh-cut FVH industry, based on the fit-for-purpose concept for process water, the available industrial data and the statistical analyses (Section 3.3), it is concluded that each FBOp should generate data to support the selection of the specific microbial indicators and their thresholds (performance standards) depending on the specific handling and processing operation (including the reasonable range of variability of the operating conditions) and the intended use of the FVH among others.

As previously mentioned, the industrial data set collected through EFSA's outsourced activities (Gil et al., 2025) did not identify clear associations between physico-chemical parameters and microbial data (Section 3.3.2), making it difficult to select the most useful physico-chemical indicators and valid performance standards to be validated. This selection needs to be done based on the scientific literature and/or model simulations (see Section 3.7).

(D) Data collection in the validation study

Important for a validation study is to take the variability of the processing operation during the production period (e.g. day, week or month) into account. The variability can be covered by designing and running a validation assay for different independent trials. Ideally, a minimum '3x3 approach' should be followed for each processing operation in post-harvest processing where water is applied:

- 3 operational cycles (on different days, weeks or months, e.g. including different seasons or different conditions at harvest (rainy or dry weather)) and
- 3 time points within the operational cycle (start, middle and end of operational cycle).

As such, 9 data sets should be collected per sampling location where a water sample is taken.

Appendix F shows a recording template table for a validation study of water used in post-harvest handling and/or processing operations.

Sampling methods, sample treatment (e.g. filtration is only done in case of low contaminated water/ice-making water/cooling water) and analytical procedures should follow standardised protocols (i.e. ISO methods when available, validated tests). Calibrated and verified sensors and devices should be used (see Section 3.8) (EFSA BIOHAZ Panel, 2023). All the conditions used and the results obtained in each validation trial should be systematically recorded (Appendix F) and summarised in the validation report. The information on key parameters should show the actual boundaries of the validated operating conditions, which will eventually be considered to set the water treatment conditions for the specific operation, allowing the maintenance of the quality of the process water in routine productions.

3.7 | Operational monitoring of the intervention measures

Operational monitoring is the systematic and continuous observation that allows real-time information from the physicochemical parameters of the process water identified as critical during the validation study.

As shown in Section 3.3.2, none of the industrial cases included in EFSA's outsourced activities (Gil et al., 2025) could be used as illustrative for operational monitoring put in place. Therefore, recommendations for operational monitoring are based on the following:

a) The scientific literature, critical physico-chemical parameters that should be used in the operational monitoring, including the residual concentration of the disinfectant, organic matter indicators (e.g. UV absorbance 254 nm), depending on the type of disinfectant and the pH of the process water (e.g. chlorine-based disinfectants) and water temperature. The analytical procedures recommended for each parameter were described in the previous Part 1 Opinion (EFSA BIOHAZ Panel, 2023).
b) The model simulations included in Section 3.4 showed that, for example, adjustment of specific physico-chemical parameters (e.g. pH and temperature) is necessary for the efficacy of the water disinfection treatment in case of the use of chlorine-based chemicals (SG 2.2 and SG 4).

Examples of critical physico-chemical parameters to take into consideration when defining operational monitoring are demonstrated in Section 3.4. However, the specific parameters and thresholds need to be determined as a result of the validation study on a case-by-case basis.

All the results obtained during the operational monitoring should be systematically recorded (Section 3.9) and periodically reviewed as part of the verification procedures (Section 3.8). Appendix G shows an example of a recording template table for operational monitoring.

If the thresholds of the operational monitoring parameters are exceeded, corrective actions to correct the production process need to be taken. Examples of such actions are: (i) complete removal of the water in the tank/processing line, (ii) increase of the water replenishment frequency or rate, (iii) correction of the dose of the disinfectant applied or mode of addition (e.g. continuous vs. discrete) and, (iv) increase of the dose of the acid (e.g. phosphoric acid) to lower the pH. As the production process is no longer under control, the microbiological quality of the process water can no longer be guaranteed. Therefore, the microbiological quality and the acceptability of the production batch of fresh-cut FVH need to be further evaluated in the frame of the overall food safety management system (EFSA BIOHAZ Panel, 2017).

3.8 | Verification of the water management plan

As described in Opinion Part 1, verification is conducted periodically to check if the microbiological quality of the process water is achieved by the validated and monitored operating conditions. Together with reviewing/checking/auditing the monitoring records and the calibration status of measuring devices, verification typically includes the microbiological testing (for selected microbial indicators after the validation study) of the process water, which can be carried out by the FBOps and/or by an independent authority (e.g. external laboratory).

As a starting point for the verification, a monthly sampling of water applied in each step of the process could be recommended (e.g. 10 process steps where water is applied × 12 samples/year = 120 outcomes of the microbiological quality of the water per year analysed for the selected microbial indicator group). The frequency of verification for each processing line and/or operation can be decreased with the accumulation of satisfactory results shown by the trend observation and analysis.

In the EU Commission Notice (2017/C 163/01) on guidance document on addressing microbiological risks in fFVs at primary production through good hygiene, including post-harvest operations at the place of such production (Annex II), a sampling frequency scheme for water used in primary agricultural production, i.e. high (one per month), medium (twice a year) and low (once a year) is set as example, which may be modified based on the risk assessment of each farm. Tools for conducting such assessments are also provided in Annex II and III. However, no recommendations were provided regarding water management plans, and particularly, how to validate, monitor and verify the implemented intervention strategies neither at primary production stage nor at processing facilities.

The same sampling and analytical methods used in the validation study should be used for verification purposes, following standardised protocols (i.e. ISO methods or validated alternatives when available). Calibrated and verified sensors and devices shall be used.

Apart from the timely intervention when an outcome is not in line with the expected, desired or set parameter (e.g. target pH, target UV absorbance at 254 nm, target residual chlorine concentration) in the operational monitoring, a trend observation (presentation of collected data in a histogram, figures, etc., to retrieve trends) and trend analysis (statistical analysis to detect significant differences) also needs to be done to identify potential systemic issues in the frame of the verification. Table 6 gives an overview of the frequency of trend observations and trend analyses based on the data collected to verify the water management plan.

TABLE 6 Proposal of frequency for trend observation and trend analysis in the frame of verification of a water management plan for the fresh-cut FVH sector.

Type of record	Trend observation	Trend analysis
Operational monitoring data (online, inline and/or offline)	Every month	Twice a year
Calibration of analytical sensors, probes, etc. (belonging to preventive measures)	Twice a year	Once a year
Validation	In case of revalidation	–
Verification	Monthly	Twice a year

In case the verification outcomes are not performing according to the set performance standards of the microbiological indicators, the quality of the process water is not in compliance with the required standard. In this case, the water management plan needs to be reviewed and revalidated (see Opinion 1).

3.9 | Record keeping and review of the water management plan

The outcomes of all records collected for the preventive measures, validation, operational monitoring and verification shall be established in the FBOp documentation systems.

Examples of record-keeping data sheets are given in Appendices F and G.

4 | CONCLUSIONS

This opinion only covers TOR 1.1., as sub-TOR from TOR1 because industrial data was unavailable to provide further insights beyond what has been included in Part 1 Opinion on the other sub-TORs from TOR1. However, all the sub-TORs from TOR2 and TOR3 were addressed.

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

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 ffFVH?

Industrial data covering 19 scenarios for the fresh-cut FVH sector obtained within the framework of EFSA's outsourced activities indicated that:

- Levels of potential microbial indicators in the process water were variable. They ranged from <0 (below LOD) to 7.3 $\log_{10}$ CFU/100 mL for TBC, from <0 (below LOD) to 2.9 $\log_{10}$ CFU/100 mL for *E. coli* and from <0 (below LOD) to 4.2 $\log_{10}$ CFU/100 mL for *Listeria* spp.
- The hazardous events linked to the handling and/or processing operations using water for the fresh-cut FVH sector include: (i) incomplete removal of the water and/or poor cleaning and disinfection in between operations, (ii) the use of the same water to wash large volumes of product during the operational cycle without a well-managed intervention strategy and (iii) nutrient-rich water due to the release of nutrients after the cutting activities.
- Bacterial pathogens were detected in 50 out of 456 water samples analysed, belonging to 5 out of 19 scenarios. *Salmonella* spp. was the most frequently detected pathogen, while STEC was found in 1 sampling occasion. *L. monocytogenes* and *E. coli* O157:H7 were not detected in any of the water samples. All positive findings of bacterial pathogens were from scenarios not applying water disinfection.
- The levels of the assessed microbial indicators (by aggregating observations within each sampling visit) were higher in process water positive for enteric pathogens than in process water in which enteric pathogens were not detected. When TBC were above 10^5 CFU/100 mL, TC above 10^4 CFU/100 mL, or *E. coli* or *Listeria* spp. were detected (i.e. above 1 CFU/100mL),

the odds of detecting enteric pathogens[17] was more than 100-fold the odds of detecting enteric pathogens when the microbial indicators were below these thresholds.
- When applying a multivariable logistic regression model that accounts for the hierarchical structure of the complete data set using random effects, the detection of pathogens in process water is influenced by multiple variables (i.e. specific combination of FVH product, type of operation, operational conditions, etc.). This results in a significant random effect primarily associated with the scenario. The variability of detection of *Salmonella* spp. is mainly due to the scenario as random effect.
- The suitability of any potential microbial indicator for verification purposes within the water management plan should be validated under the specific operational conditions of each FBOp.

TOR2 aims 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 ffFVH, taking into account their impact on the physiological state of the microbiological hazards present in the water***.

Industrial data covering 19 scenarios for the fresh-cut FVH sector obtained within the framework of EFSA's outsourced activities showed that:

- Points of attention were identified regarding prerequisite programme practices are needed to avoid microbiological contamination and proliferation in process water. The following preventive measures should be prioritised:
  - PRP 1: Infrastructure and fit-for-purpose building and equipment
  - PRP 2: Cleaning and disinfection
  - PRP 4: Technical maintenance and calibration
  - PRP 8: Water and air control
  - PRP 9: Personnel (hygiene, health status and training)
  - PRP 12: Working methodology
- When no water disinfection treatment was applied, the microbiological quality of process water did not achieve a fit-for-purpose microbiological quality. It should be noted that no FBOp applied water replenishment. When water disinfection treatments were applied, physico-chemical parameters indicated that the water management system was not adjusted to achieve a fit-for-purpose microbiological quality. These results stress the need to implement validation, operational monitoring and verification in industrial settings to achieve a proper water management in this sector.
- According to the mathematical model simulations based on chlorine-based disinfectants:
  - Adequate water management requires continuous monitoring and adjustment of physico-chemical parameters, such as the residual concentration of disinfectant (e.g. FC concentration > 3–5 mg/L) and pH (6.0–6.5) to maintain the microbiological quality of the process water within established ranges.
  - Disinfection is needed to maintain the fit-for-purpose microbiological quality of the process water, whereas water replenishment applied alone cannot avoid accumulation of microorganisms in process water when applied at realistic feasible rates. Water replenishment combined with water disinfection treatments facilitates the management of the microbiological water quality, reducing the impact of the organic matter on the efficacy of chlorine.
- When the levels of VBNC bacterial cells were quantified in process water, it was observed that in the case of TBC, levels of VBNC cells were always higher than the culturable cells. Similar results were also found in VBNC cells of TC. This indicates a low efficacy of the intervention strategies to avoid the induction of VBNC cells.

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

- The validation study of the efficacy of the intervention measures should be performed by each FBOp to support the selection of the specific indicators and their thresholds (performance standards), considering the fit-for-purpose water concept depending on the specific handling and processing operation.
  - A reasonable range of variability of the operating conditions and the intended use of the FVH among others should be considered.
  - FBOps could use the predictive mathematical model made available in a user-friendly tool (https://r4eu.efsa.europa.eu/app/WaterManage4You) for a preliminary in-silico study of the validation assay to evaluate whether the foreseen conditions of the water treatment could be suitable to maintain the fit-for-purpose microbiological quality of process water.

[17] 48 out of 50 detected enteric pathogens were *Salmonella* spp.-positive samples, with all 48 agglutination-confirmed.

- Operational monitoring is the systematic and continuous observation that allows real-time information on the physico-chemical process parameters identified as critical during the validation study.
  - Critical physico-chemical parameters to be monitored should include but are not limited to organic matter (measured as UV absorbance 254 nm), residual concentration of the disinfectant, pH (depending on the type of disinfectant) and temperature.
  - Corrective actions are required when the established thresholds for physico-chemical parameters are not met.
- Verification is conducted periodically to check if the microbiological quality of the process water is achieved by different activities, such as review of monitoring records and the calibration status of measuring devices, in combination with sampling of the process water for analysing the selected microbial indicators (e.g. TBC, TC, *E. coli* and/or *Listeria* spp.).
  - The frequency of verification can be decreased depending on satisfactory results shown by the trend observation and analysis.
  - In case the verification outcomes are not performing according to the set performance standards of the microbiological indicators, the process water does not conform to fit-for-purpose microbiological quality, and the water management plan needs to be reviewed and/or revalidated.
- Record keeping and review of the water management plan is critical. The outcomes of all records collected for the preventive measures, validation study, operational monitoring and verification shall be established in the FBOp documentation systems.

5 | RECOMMENDATION

- Relevant stakeholders can use the developed mathematical model for their FVH sector to understand the impact of certain parameters and intervention measures on the process water quality, using specific data generated in their industrial settings.
- To further investigate the efficacy of non-chemical water disinfection methods, either alone or in combination with existing practices, in industrial settings.
- In view of the increasing water scarcity it is recommended to explore the implementation of all possible water treatments to enhance water reuse.
- To further explore the association between potential microbial indicators and the detection of pathogens in process water.
- To evaluate chemical hazards linked to the use of water disinfection treatments aiming to maintain the fit-for-purpose quality of process water.

ABBREVIATIONS

AQ	Assessment question
BIOHAZ	Biological Hazards
CaClOH	calcium hypochlorite
CFU	colony forming units
COD	chemical oxygen demand
EC	electrical conductivity
ECDC	European Centre for Disease Prevention and Control
FAO	Food and Agriculture Organization of the United Nations
FBOp(s)	food business operator(s)
FC	free chlorine
ffFVH	fresh and frozen fruit, vegetables and herbs
FVH	fruit, vegetables and herbs
GHP	good hygienic practices
GMP	good manufacturing practices
H_2O_2	hydrogen peroxide
HACCP	Hazard Analysis Critical Control Point
HAV	hepatitis A virus
HOCl	hypochlorous acid
ISO	International Organization for Standardization
LOD	limit of detection
MI	microbial indicator
NaClOH	sodium hypochlorite
OPRP	operational pre-requisite programme
OR	odds ratio

ORP	oxidation–reduction potential
PAA	peroxyacetic acid
PCA	principal component analysis
PCR	polymerase chain reaction
pH	potential of hydrogen
PRP	pre-requisite programme
RTE	ready-to-eat
RT-qPCR	quantitative real time polymerase chain reaction
SG	scenario group
SO	Scientific opinion
SOP	standard operating procedures
STEC	Shiga toxin-producing *Escherichia coli*
T	temperature
TBC	total bacterial count
TC	total coliforms
TDS	total dissolved solids
Th	threshold
ToR(s)	terms of reference
TSS	total soluble solids
VBNC	viable but non-culturable
WD	water disinfection
WHO	World Health Organization
WMP	water management plan
WR	water replenishment

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APPENDIX A

Uncertainty analysis

TABLE A1 Potential sources of uncertainty related to the industrial data set collected through EFSA's outsourced activities (Gil et al., 2025) for fresh-cut FVH processing and its use.

Source or location of the uncertainty	Nature or cause of the uncertainty
A limited number of EU FBOps (8) who contributed to EFSA's outsourced activities are active in the fresh-cut FVH sector	The FBOps belong to 3 Member States, resulting in an uncertainty that not all practices in processing fresh-cut FVH across Europe were included in the available data set
A limited number of scenarios (19) in the data set collected through EFSA's outsourced activities for fresh-cut FVH resulted in a limited range of commodities (11 types) and a limited selection of the potential use of water in post-harvest processing activities and water management/treatment possibilities	This results in an uncertainty that not all practices in the processing of fresh FVH across Europe were included in the available data set. The scenarios considered also applied a limited number of interventions, i.e. all were based on the use of chemicals to disinfect the water. Therefore, data was lacking for other interventions, such as water replenishment (or refreshment) or physical water disinfection technologies
The data set collected through EFSA's outsourced activities was assembled in a certain time period (4 April 2022 to 4 April 2024) with a fractionated data collection (1 or 2 sampling visits per scenario)	The nature of the collected data can be uncertain as no full picture of the specific situation in a particular company with a certain water management practice was collected
The data set collected through EFSA's outsourced activities harbours an uncertainty related to the distribution of the sampling time points, which do not always represent the full duration of the operational cycle	For example, 6 sampling time points per sampling visit per scenario set more in the beginning or later in an operational cycle. Therefore, an unequal accumulation of the microbial load of the process water can be expected, or sampling time point 0 may not correspond to the start of the operational cycle
Norovirus and indicator viruses (bacteriophages) were only analysed at the last sampling time point of each sampling visit of an operational cycle	Furthermore, norovirus was analysed by RT-qPCR, showing the presence of norovirus genomes, and for Norovirus GI, also after the addition of PMAxx, which indicates the presence of intact virions, but not their infectivity
The methods used in EFSA's outsourced activities to confirm presumptive *Salmonella* spp. and *L. monocytogenes* colonies were different (PCR vs. latex agglutination (*Salmonella*) and PCR vs. carbohydrate fermentation testing (*L. monocytogenes*)). These confirmation methods are not reference methods and are considered alternative methods	The positive samples confirmed using the alternative methods cannot be considered equivalent. For *Salmonella*, none of the methods used can be considered validated for process water samples, but both methods confirm suspect colonies (no serogroup or serotype identification). Therefore, results presented in the scientific opinion always specify if these were generated by PCR-confirmation, agglutination-confirmation or carbohydrate fermentation testing-confirmation, respectively
There were also sources of uncertainties linked to the predictive model used to illustrate the efficacy of different intervention strategies	The uncertainty sources associated with the model structure (i.e. suitability of the model to simulate the process) are considered to have a lower impact on the outcome than the uncertainty in the data used to fit the model. This is further elaborated in the Part 2 Opinion (EFSA Panel on Biological Hazards, 2025a)

Abbreviations: FBOp, food business operator; FVH, fruit, vegetables and herbs; PCR, polymerase chain reaction; RT-qPCR, quantitative real time polymerase chain reaction.

APPENDIX B

Industrial scenarios for the fresh-cut FVH sector in EFSA's outsourced activities (Gil et al., 2025; Table B1)

TABLE B1 Overview of the industrial scenarios for the fresh-cut FVH sector (EFSA outsourced activities – Gil et al., 2025).

Scenario ID	Water source	Handling/processing operation	Disinfectant type	Food product
30	Well water	Washing	None	Shredded carrots
31	Well water	Washing	None	Curly endive and radicchio
32	Municipal water- Well water	Washing	None	Baby leaves
33	Well water	Washing	None	Parsley
34	Municipal tap water	Washing	None	Salad mix with carrots
35	Municipal tap water	Pre-washing	Electrolysed water	Tomatoes/cucumber
36	Municipal tap water	Washing	Electrolysed water	Tomatoes/cucumber
37	Municipal tap water	Washing	Sodium hypochlorite	Diced onion
38	Well water	Washing	PAA	Diced onion
39	Municipal tap water	Washing	Sodium hypochlorite	Carrot sticks
40	Municipal tap water	Washing	Calcium hypochlorite	Fresh-cut lettuce
41	Municipal tap water	Washing	Sodium hypochlorite	Fresh-cut lettuce
42	Well water	Washing	Chlorine gas	Fresh-cut lettuce
43	Municipal tap water	Pre-washing	Sodium hypochlorite	Shredded lettuce
44	Municipal tap water	Pre-sorting	Sodium hypochlorite	Shredded lettuce
45	Municipal tap water	Washing	Sodium hypochlorite	Baby leaves
46	Municipal tap water	Washing	Calcium hypochlorite	Baby leaves
47	Municipal tap water	Washing	Sodium hypochlorite	Baby leaves
48	Municipal tap water	Washing	Sodium hypochlorite	Salad mix

Abbreviations: FVH, fruit, vegetables and herbs; PAA, peroxyacetic acid.

APPENDIX C

Pathogen-positive findings in process waters of fresh-cut FVH (Table C1)

TABLE C1 Pathogen-positive process waters characterised by food product, water source, disinfectant type, sampling visit, sampling time point and mass of fresh-cut FVH processed in the same water (*Source*: EFSA outsourced activities – Gil et al., 2025).

Scenario ID	Food product	Handling/ processing operation	Water source	Disinfectant type	Sampling visit number	Sampling time point	Sampling time (min)	Product mass processed (kg)	Pathogen findings[a]
30	Shredded carrots	Washing	Well water	No water treatment	1	1	0	0	*Salmonella*
30	Shredded carrots	Washing	Well water	No water treatment	1	2	25	644	*Salmonella*
30	Shredded carrots	Washing	Well water	No water treatment	1	3	50	1035	*Salmonella*
30	Shredded carrots	Washing	Well water	No water treatment	1	4	75	1875	*Salmonella*
30	Shredded carrots	Washing	Well water	No water treatment	1	5	100	2555	*Salmonella*
30	Shredded carrots	Washing	Well water	No water treatment	2	1	0	0	*Salmonella*
30	Shredded carrots	Washing	Well water	No water treatment	2	2	50	353	*Salmonella*
30	Shredded carrots	Washing	Well water	No water treatment	2	5	266	2627	*Salmonella*
30	Shredded carrots	Washing	Well water	No water treatment	2	6	293	2627	Norovirus[b]
31	Curly endive and radicchio	Washing	Well water	No water treatment	1	2	36	116	STEC
31	Curly endive and radicchio	Washing	Well water	No water treatment	1	3	72	400	*Salmonella*
31	Curly endive and radicchio	Washing	Well water	No water treatment	1	4	108	600	*Salmonella*
31	Curly endive and radicchio	Washing	Well water	No water treatment	1	5	144	859	*Salmonella*
31	Curly endive and radicchio	Washing	Well water	No water treatment	1	6	180	1231	*Salmonella*
31	Curly endive and radicchio	Washing	Well water	No water treatment	2	1	0	0	*Salmonella*
31	Curly endive and radicchio	Washing	Well water	No water treatment	2	2	40	426	*Salmonella*
31	Curly endive and radicchio	Washing	Well water	No water treatment	2	4	144	987	*Salmonella*
31	Curly endive and radicchio	Washing	Well water	No water treatment	2	5	185	1308	*Salmonella*
31	Curly endive and radicchio	Washing	Well water	No water treatment	2	6	235	1710	Norovirus[c]
32	Baby leaves	Washing	Municipal + well water	No water treatment	1	1	0	0	STEC
32	Baby leaves	Washing	Municipal + well water	No water treatment	2	1	0	0	*Salmonella*
32	Baby leaves	Washing	Municipal + well water	No water treatment	2	2	90	124.5	*Salmonella*
32	Baby leaves	Washing	Municipal + well water	No water treatment	2	6	510	1308.22	Norovirus[c]
33	Parsley	Washing	Well water	No water treatment	1	1	0	0	*Salmonella*
33	Parsley	Washing	Well water	No water treatment	1	2	12	20	*Salmonella*
33	Parsley	Washing	Well water	No water treatment	1	5	48	80	*Salmonella*
33	Parsley	Washing	Well water	No water treatment	1	6	72	130	*Salmonella*

TABLE C1 (Continued)

Scenario ID	Food product	Handling/ processing operation	Water source	Disinfectant type	Sampling visit number	Sampling time point	Sampling time (min)	Product mass processed (kg)	Pathogen findings[a]
33	Parsley	Washing	Well water	No water treatment	2	1	0	0	*Salmonella*
33	Parsley	Washing	Well water	No water treatment	2	3	27	70	*Salmonella*
33	Parsley	Washing	Well water	No water treatment	2	4	39	90	*Salmonella*
33	Parsley	Washing	Well water	No water treatment	2	5	51	130	*Salmonella*
33	Parsley	Washing	Well water	No water treatment	2	6	90	160	*Salmonella*, Norovirus[c]
34	Salad mix with carrots	Washing	Municipal tap water	No water treatment	1	2	27	221	*Salmonella*
34	Salad mix with carrots	Washing	Municipal tap water	No water treatment	1	4	75	528	*Salmonella*
34	Salad mix with carrots	Washing	Municipal tap water	No water treatment	1	6	105	792	*Salmonella*
34	Salad mix with carrots	Washing	Municipal tap water	No water treatment	2	3	86	176	*Salmonella*
34	Salad mix with carrots	Washing	Municipal tap water	No water treatment	2	4	111	352	*Salmonella*
34	Salad mix with carrots	Washing	Municipal tap water	No water treatment	2	6	156	681	*Salmonella*
37	Diced onions	Washing	Municipal tap water	Chlorine	2	6	405	4173	Norovirus
41	Fresh-cut lettuce	Washing	Municipal tap water	Chlorine	1	6	570	2100	Norovirus[c]
41	Fresh-cut lettuce	Washing	Municipal tap water	Chlorine	2	6	540	4909	Norovirus
43	Shredded lettuce	Pre-washing	Municipal tap water	Chlorine	2	6	390	11,248	Norovirus
44	Shredded lettuce	Washing	Municipal tap water	Chlorine	2	6	390	11,248	Norovirus
47	Baby leaves	Washing	Municipal tap water	Chlorine	1	6	570	7000	Norovirus
47	Baby leaves	Washing	Municipal tap water	Chlorine	2	6	540	1388	Norovirus[c]

Abbreviation: FVH, fruit, vegetables and herbs.

[a]Confirmation of *Salmonella* was performed by agglutination testing. STEC was confirmed by PCR. The presence of norovirus GI and GII was detected by RT-qPCR.

[b]Intact capsid was assessed by RT-qPCR after PMAxx pretreatment and RNA extraction in Norovirus GI-positive samples.

[c]48 out of 50 detected enteric pathogens were *Salmonella* spp.-positive samples, with all 48 agglutination-confirmed.

APPENDIX D

Relationship between the occurrence of potential microbial indicator groups above a given threshold and the detection of enteric pathogens (*Salmonella* spp. or STEC) (Table D1)

TABLE D1 Odds ratio (OR) for the detection of **enteric pathogens** (agglutination-confirmed *Salmonella* and/or PCR-confirmed STEC)[d] process water from the fresh-cut FVH for different thresholds of TBC, TC, *E. coli* and *Listeria* spp. as potential microbial indicator groups (results analysed aggregating by sampling visit). The OR calculated using the complete data set for all sectors (fresh-whole, fresh-cut and frozen FVH) are also included for comparison.

Bacterial indicator group and its threshold	Data concerning fresh-cut FVH sector			Complete data set for all sectors (fresh-whole, fresh-cut and frozen FVH)		
	OR	95% CI	Significance level (p)	OR	95% CI	Significance level (p)
Total bacterial count threshold (CFU/100 mL)						
10^4	**27.0**	2.9–253.6	0.004	–[a]		
10^5	**117.0**	9.4–1450.3	< 0.001	–[a]		
10^6	–[a]			**12.2**	2.6–58.3	0.002
Total coliform threshold (CFU/100 mL)						
10^2	–[b]			**8.6**	1.1–68.7	0.042
10^3	**54.0**	5.3–550.3	< 0.001	**12.7**	1.6–101.4	0.016
10^4	**117.0**	9.4–1450.3	< 0.001	**5.0**	1.3–19.4	0.019
E. coli threshold (CFU/100 mL)						
1[c]	**108.0**	8.6–1351.5	< 0.001	**7.4**	1.6–35	0.012
10	–[a]			**11.7**	2.4–55.7	0.002
100	–[a]			**26.0**	5.3–127.4	< 0.001
***Listeria* spp**. threshold (CFU/100 mL)						
1[c]	**117.0**	9.4–1450.3	< 0.001	**5.8**	1.2–27.6	0.026
10	–[a]			**11.2**	2.3–53.2	0.003
100	–[a]			**5.2**	1.6–17.1	0.007

Note: Bold indicates statistically significant differences.

Abbreviations: CFU, colony forming unit; CI, confidence interval; FVH, fruit, vegetables and herbs; PCR, polymerase chain reaction; STEC, Shiga toxin-producing *Escherichia coli*; TBC, total bacterial count; TC, total coliforms.

[a]OR could not be calculated because samples with enteric pathogens not detected did not show microbial indicators above the threshold.

[b]OR could not be calculated because enteric pathogens were not detected in any sample with microbial indicators below the threshold.

[c]Limit of detection.

[d]48 out of 50 detected enteric pathogens were *Salmonella* spp.-positive samples, with all 48 agglutination-confirmed.

APPENDIX E

Model parameters and variables used to simulate the different scenarios groups (SGs)

TABLE E1 Input parameter values for model simulations of all the scenario groups (SGs) of the fresh-cut FVH sector.

Parameter	Value	Units	Description	Source for the value (reference)
α	7.66	1/(mg-FC/L·min)	Inactivation rate coefficient	Estimated value from TBC data collected through EFSA's outsourced activities (Gil et al., 2025)
β	7.81×10^{-4}	1/(mg-COD/L·min)	Rate constant of reaction FC and COD	Estimated value (Gil et al., 2025)
γ	8.16	mg-COD/mg-FC	Yield coefficient COD of reaction FC and COD	Estimated value (Gil et al., 2025)
λ	1.70×10^{-3}	1/min	Natural decay of FC	Retrieved from Abnavi et al. (2021)
n	1	–	Exponent in Hill equation	Assumed (Gil et al., 2025)
K_m	2.13×10^{3}	mg-COD/L	Protective COD effect	Estimated value from data collected through EFSA's outsourced activities (Gil et al., 2025)
K_X	2.33×10^{7}	CFU/(kg-product·min)	Transfer rate coefficient of bacteria from product to water	Estimated value from data collected through EFSA's outsourced activities (Gil et al., 2025)
K_{COD}	7.57×10^{2}	mg-COD/(kg-product·min)	Transfer rate coefficient of organic matter from process water to product	Estimated value from data collected through EFSA's outsourced activities (Gil et al., 2025)

Abbreviations: CFU, colony forming unit; FVH, fruit, vegetables and herbs; TBC, total bacterial count.

TABLE E2 Input data for variables in model simulations of all the scenario groups (SGs) of the fresh-cut FVH sector.

Variables	Value	Units	Description
V	2300	L	Tank water volume
τ	1	min	Contact time
P	5624	kg	Total product added
COD_i	0	mg-COD/L	Initial COD in water (mg/L)
Xw_i	0	CFU/100 mL	Initial bacterial contamination level (CFU/mL)

Abbreviations: CFU, colony forming unit; COD, chemical oxygen demand; FVH, fruit, vegetables and herbs.

APPENDIX F

Recording template table for a validation study for water used in different post-harvest handling and/or processing operations

TABLE F1 Example of a recording template table for validation of post-harvest handling and/or processing operations using water in the fresh-cut FVH sector (*fixed, **dynamic, ***in case disinfection is applied).

Post-harvest handling and/or processing operation	Date	Hour	Product and process-related parameters* (e.g. commodity, equipment information as tank volume)	Water-related parameters* (e.g. water hardness, water temperature, water addition)	Water-related parameters** Measured pH	Water-related parameters** Measured COD	Water disinfection treatment-related parameters*, *** Measured UV absorbance	Water disinfection treatment-related parameters*, *** Temperature (°C)	Water disinfection treatment-related parameters*, *** e.g. type, mode of dosing (continuous/discontinuous)	Water disinfection treatment-related parameters*** Initial concentration (mg/L)	Water disinfection treatment-related parameters*** Measured residual concentration (mg/L)	Analytical result of indicator in water (CFU/100 mL)
Transport/sorting water:	**Trial 1**	Sampling time point 1 (start)										
		Sampling time point 2 (middle)										
		Sampling time point 3 (end)										
	Trial 2	Sampling time point 1 (start)										
		Sampling time point 2 (middle)										
		Sampling time point 3 (end)										
	Trial 3	Sampling time point 1 (start)										
		Sampling time point 2 (middle)										
		Sampling time point 3 (end)										
Wash process: rinsing water												
Wash process: prewash water	…											
Wash process: washing water	….											
Dumping tank water	…											
Water applied in hydrocooling	…											

Abbreviations: CFU, colony forming unit; COD, chemical oxygen demand; FVH, fruit, vegetables and herbs.

APPENDIX G

Recording template table for operational monitoring

TABLE G1 Example of template for recording table for operational monitoring of post-harvest handling and/or processing operations using water in the fresh-cut FVH sector.

Post-harvest handling and/or processing operation	Food product	Date	Hour	Product mass (kg) being processed	Residual disinfectant concentration (mg/L)	pH	UV absorbance	Temperature (°C)	Water addition (L/min)

Abbreviation: FVH, fruit, vegetables and herbs.

ANNEX A

Excel file including the full data set collected during the sampling visits to all FBOps collaborating with the EFSA outsourced activities (Gil et al., 2025)

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

ANNEX B

Statistical analysis of the relationship between potential microbial indicator groups and pathogen detection in process water of fresh and frozen fruits, vegetables and herbs: a multivariable, logistic mixed-effect regression model

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