

# 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 5 (Frozen FVH process water management plan)

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## 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 frozen FVH sector is characterised by operational cycles between 8 and 120 h, variable product volumes and no control of the temperature of process water. Intervention strategies were limited to the use of water disinfection treatments such as peroxyacetic acid and hydrogen peroxide. Chlorine-based disinfectants were not used, and water replenishment was not observed within studied industries. The industrial data, which included 13 scenarios, were used to develop a guidance for a water management plan (WMP) for the frozen 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. 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

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

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## SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Biological Hazards (BIOHAZ) to provide a scientific opinion on the microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (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 5 Opinion is specific to the frozen 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 (FBOs) (EFSA outsourced activities – Gil et al., 2025), the main characteristics of the sector were identified. Results showed that common post-harvest handling operations include pre-washing, cooling and rinsing. The water temperature was usually not controlled and usually at room temperature (around 20°C). When water samples were collected in operations after the blanching step, the temperature of the process water was higher, reaching levels above 60°C. The water used during these operations is sourced from recycled water, followed by municipal tap water and well water. The operational cycle of some scenarios lasted about 4–5 h, while in other scenarios, operational cycles lasted 120 h. The amount of product that is processed during the operational cycle varied among the scenarios, with the most common cumulative values reported between a few tonnes (2–3) to up to 1000 tonnes. Water volumes of the water tanks were very variable, ranging between 2000 L and 5000 L, with maximum volumes of 25,000 and 40,000 L. The product-to-water contact time during operations varied from a few seconds (15–30 s) to 7–10 min, but in most of the cases, it was below 2 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 the 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 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 FBO.

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 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 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 frozen FVH sector, intervention strategies were based on water disinfection treatments using PAA and  $H_2O_2$ . Chlorine-based disinfectants were not used by any of the industrial cases. In this sector, one-third of the industrial scenarios (4/13) did not apply any intervention strategy aiming to maintain the fit-for-purpose microbiological quality of the process water.

In order to assist FBOs 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 developed 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 FBO 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, etc.) 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 frozen FVH sector**, the most relevant microorganisms identified as hazards in process water included *Salmonella* spp. and *L. monocytogenes*. However, there was only one positive finding of STEC. In addition to the common hazardous events listed above, the use of contaminated water sources and the improper cooling of process water after the blanching step have been identified as additional hazardous events.

Regarding a potential relationship between the detection of enteric pathogens and levels of microbial indicators, based on the obtained results, no statistically significant OR was obtained (as confirmed by the 95% CI) except for *E. coli* counts and enteric pathogens, with OR= 11 (95% CI: 1.1–109.7,  $p < 0.05$ ) at the threshold levels of 1 and 10 CFU/100 mL. This highlights the lack of relationship between most of the assessed microbial indicator groups (type and levels) and the detection of *L. monocytogenes* in process water.

When the above-mentioned logistic regression model was applied, it was observed that the odds of detection of *Salmonella* would increase by a factor of about 2 for each  $\log_{10}$ -unit increase of the concentration of *E. coli*. However, 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, etc.), 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 FBO.

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 fffFVH is an important consideration and should be controlled by an operational prerequisite program (oPRP) activity 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 fffFVH. 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 fffFVH 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 fffFVH, 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 fffFVH 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'<sup>1</sup> 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<sup>2</sup> 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) 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 (e.g. FAO/WHO, 2019; Gombas et al., 2017), if water is contaminated during washing and then used to process large quantities of fffFVH, 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.

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

<sup>1</sup>Council Directive 98/83/EEC of 3 November 1998 on the quality of water intended for human consumption (OJ L 330, 5.12.1998, p. 32–54) 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, p. 1–62).

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

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 (FBOs). 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). 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):

**TOR 1 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?

**TOR 2 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?

**TOR 3 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?

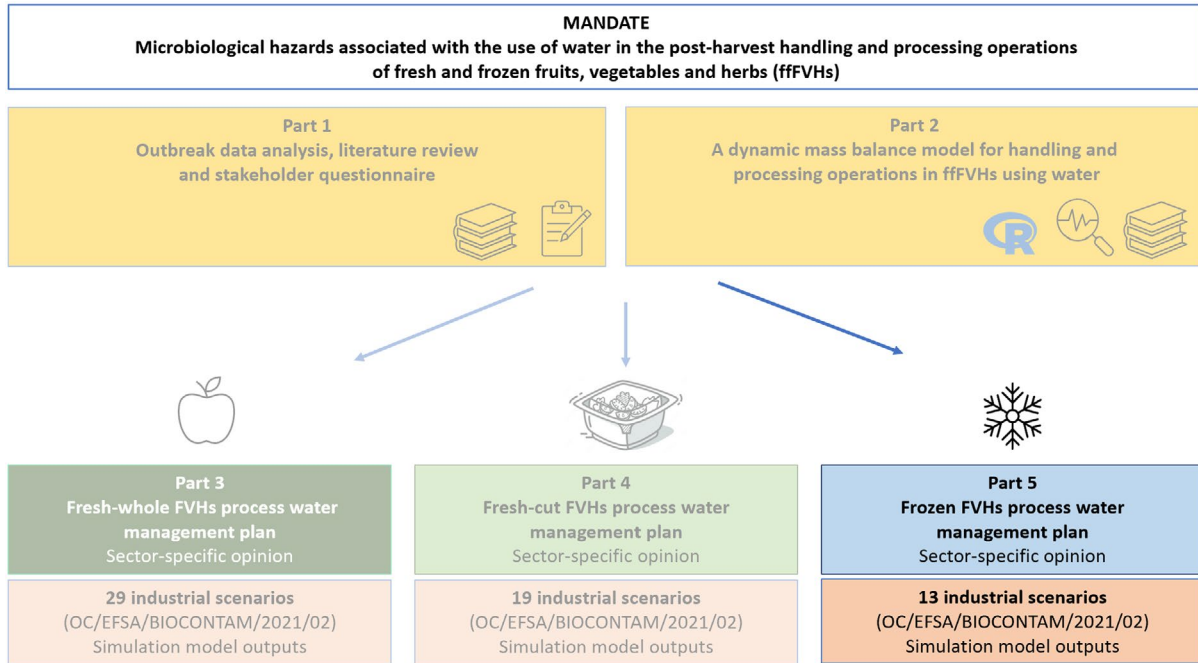
**TOR 3.2:** Which relevant parameters can be used to monitor the appropriate microbiological quality requirements of water that is being used during different post-harvest handling and processing operations for fffVH?

## 1.2 | Additional information

The Mandate on Microbiological Hazards in Water Use during Postharvest 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 fFVH. 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 (fFVH)' (including EFSA outsourced activities – Gil et al., 2025).

### 1.3 | Interpretation of the Terms of Reference (if appropriate)

This scientific opinion for the frozen FVH sector focuses on the evaluation of microbiological hazards that might be present in the process water used in different 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-whole FVH together with the strategies that can be applied by the FBOps to keep the microbiological quality of the process water according to its use, i.e., fit-for-purpose quality.<sup>3</sup> 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 frozen FVH sector-specific guidance focuses on all the activities previously described in the Part 1 EFSA scientific opinion (EFSA BIOHAZ Panel, 2023), with an emphasis on evidence generated from the EFSA outsourced activities described in Gil 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.

<sup>3</sup>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).



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. 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.  $\geq 50\%$  of the total volume, 1 m<sup>3</sup>/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 in the frozen FVH sector. Only water refilling is done as described above.

3) **Complete removal:** In the frozen 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 frozen FVH sector, the duration of the operational cycle varied from 8 to 120 h. For shorter cycles, samples were taken at regular intervals, such as every 2 h for an 8-h operational cycle (0, 2, 4, 5, 6 and 8 h). For longer cycles, sampling time points were adjusted accordingly to ensure that data were collected more or less evenly distributed throughout the entire process. For example, in one scenario with a 120-h operational cycle, samples were taken at 0, 18, 27, 47, 99 and 115 h. 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 frozen FVH industry settings and of relevance 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 coliform count

(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 food-borne 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 frozen 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 were 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 frozen 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 13 industrial scenarios of the frozen FVH sector, were analysed in three steps.

First, results of the levels of potential microbial indicators (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 pathogens were detected and not detected were graphically represented through box-and-whisker 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 frozen 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 ( $10^4$ , $10^5$ and $10^6$ CFU/100 mL)
TC	2, 3 and 4 $\log_{10}$ CFU/100 mL ( $10^2$ , $10^3$ and $10^4$ CFU/100 mL)
<i>E. coli</i>	0, 1 and 2 $\log_{10}$ CFU/100 mL (1, 10 and 100 CFU/100 mL)
<i>Listeria</i> 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 coliform count.

Secondly, the relationship between the occurrence of potential microbial indicators above the thresholds indicated in Table 1 and the detection of pathogens (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 time points and replicated samples within each visit. The odds ratio<sup>4</sup> (OR) of detecting the pathogen depending on the threshold (Th) of the microbial indicator (MI) was calculated as:

$$OR = \frac{\text{Odds MI} \geq \text{Th}}{\text{Odds MI} < \text{Th}} = \frac{a/b}{c/d}$$

where:

*a* is the number of visits in which the pathogen was **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.

<sup>4</sup>Odds measure how likely the detection of the pathogen is, and is calculated when the indicator is below and above the given threshold.

*b* is the number of visits in which the pathogen was **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 pathogen was **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 pathogen was **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<sup>5</sup> 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 detecting 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<sub>10</sub> CFU/100 mL) on the detection of the relevant pathogen (*Salmonella* or *L. monocytogenes*) 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, and for *L. monocytogenes* were only fresh-whole FVH and frozen FVH since no positive results were observed in fresh-cut 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.

When modelling the detection of *L. monocytogenes*, an additional fixed factor was included to consider whether the detection of the pathogen was confirmed by PCR or carbohydrate fermentation testing. Two different models for *Salmonella* and four for *L. monocytogenes* 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 and microbiological parameters. A principal component analysis (PCA) was performed among microbiological and physico-chemical 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 frozen 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 A.1 in Appendix A).

<sup>5</sup>MedCalc Software Ltd. Odds ratio calculator. [https://www.medcalc.org/calc/odds\\_ratio.php](https://www.medcalc.org/calc/odds_ratio.php) (Version 22.023; accessed April 7, 2024).

### 3 | ASSESSMENT (FROZEN 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 (diced) onions, spinach, diced peppers, parsley and chives (Gil et al., 2025) (Appendix B). Traditionally, frozen FVH are not ready-to-eat (RTE) food, and they are intended to be cooked before eating. However, the changing consumer behaviour towards 'healthy' and 'convenience' meals has changed consumer perception, meaning that consumers can treat frozen vegetables as safe to eat without cooking. The FBOp decides if the product is RTE or not. In case it is not RTE, the cooking instructions need to be validated, as well as the storage/handling conditions (freezing, thawing, time/temperature conditions after thawing, etc.) (EFSA BIOHAZ Panel, 2020).

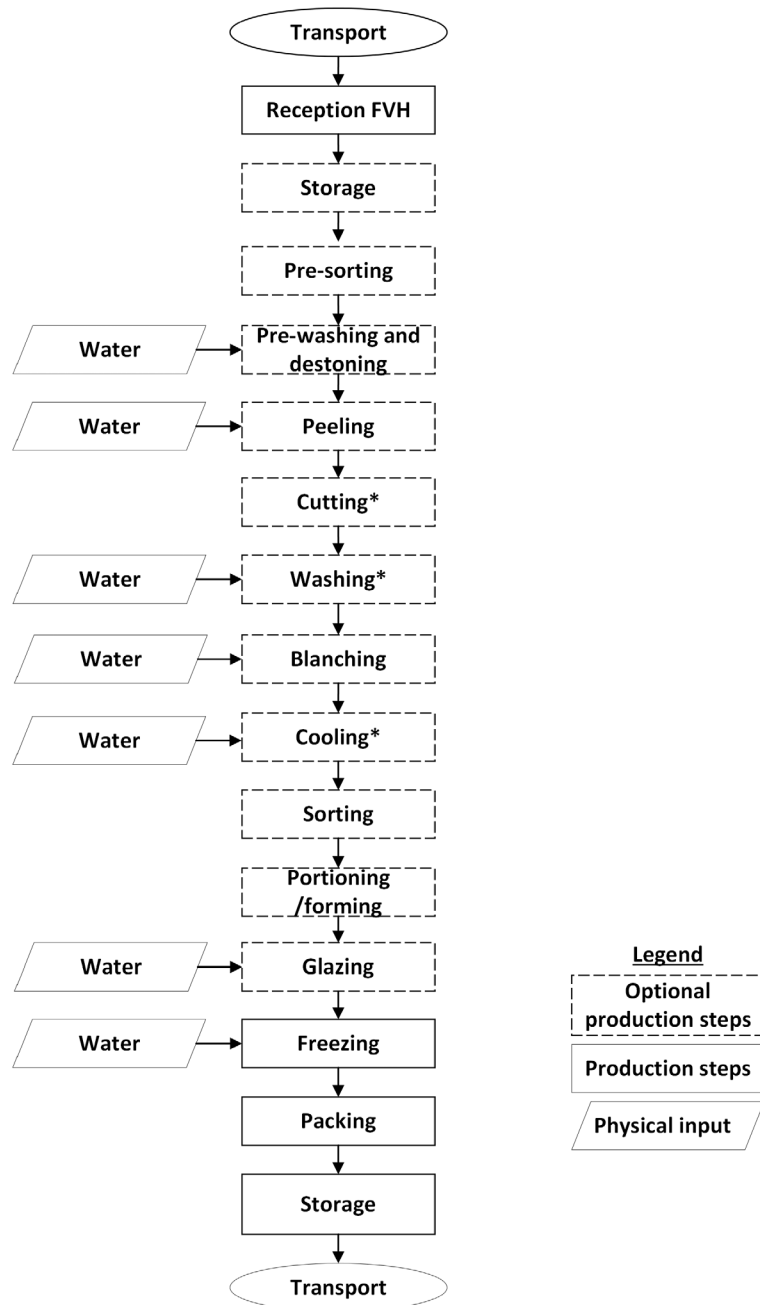
The minimum water quality requirements established for process water used in post-harvest handling and processing operations are different for RTE and non-RTE foods, based on the European Commission Notice, Annex II of EU Commission Notice (2017/C 163/01)<sup>6</sup> for details.

Handling and processing operations using water for frozen FVH have been extensively described in the Part 1 opinion. Figure 2 shows a general flow chart for the handling and processing of FVH, which includes steps where water can be used, like (pre-)washing (including paddle and flotation washing), rinsing, water transport, blanching, cooling, glazing and freezing. The EFSA outsourced activities included scenarios using water during product transport, pre-washing, washing and cooling (Appendix B).

It is relevant to mention that in the case of frozen diced peppers, water was sampled during pre-washing (Scenario ID 53) and cooling (Scenario ID 54), which occurred before cutting these products. For diced onions, water was sampled pre-washing before cutting (Scenario ID 56) and during cooling after cutting (Scenario ID 55). It should also be noted that water was sampled in FVH that underwent blanching (7 cases): in four cases, the sampling took place in operations that occurred previous to blanching (Scenarios ID 50, ID 51, ID 52 and ID 53), and in three cases, the sampling took place in an operation that occurred after blanching, i.e. cooling after blanching (Scenarios ID 54, ID 58 and ID 59).

Within the industrial cases included in the WASTHOP Tender, scenarios involving leafy greens are the most predominant commodity with seven scenarios, followed by four scenarios of bulbs and roots, and two scenarios of vegetable fruits. No scenario was included for frozen berries.

<sup>6</sup>European Commission, 2017. Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (2017/C 163/01). OJ C 163, 23.5.2017, p. 1–40.



**FIGURE 2** A general flow chart for handling and processing **frozen** fruit, 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 during EFSA's outsourced activities (Gil et al., 2025).

### 3.1.2 | Characterisation of the industrial handling and processing operations for the frozen FVH sector

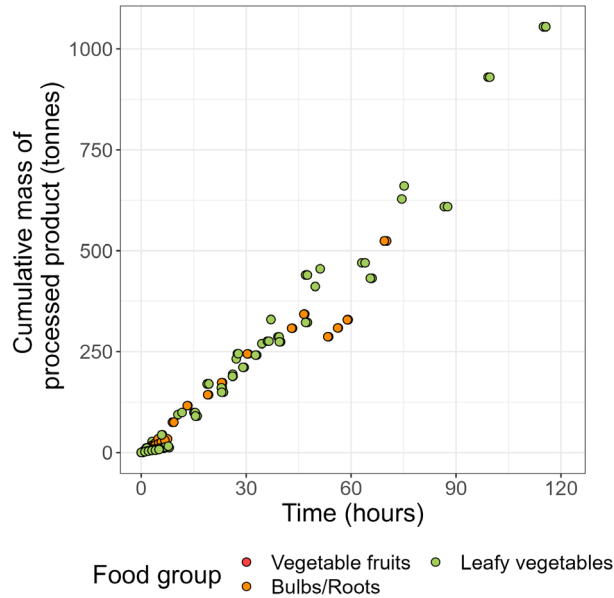
Data generated by EFSA's outsourced activities included a total of 13 industrial scenarios for frozen FVH processing from two different European countries (Gil et al., 2025). The main water source used in this sector to fill the water tanks was recycled water (5 scenarios). In four scenarios, municipal tap water has been declared as a water source, while in three scenarios well water was used. Only one scenario declared the use of municipal tap water combined with well water (Appendix B).

Among all the scenarios evaluated by the tender, four scenarios did not apply any intervention strategy aiming to maintain the microbiological quality of the fit-for-purpose process water. The rest of the scenarios used different water disinfection treatments, such as peroxyacetic acid (PAA) (5 scenarios) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (4 scenarios) (Appendix B). Chlorine-based disinfectants were not applied in any of the industrial scenarios, which could be expected as many FBOps of the frozen FVH sector stopped using chlorine in the last years as a consequence of the new Commission Regulation (EU) 2020/749 of 4 June 2020<sup>7</sup> amending Annex III to Regulation (EC) No 396/2005 of the European Parliament and of the Council

<sup>7</sup>Commission Regulation (EU) 2020/749 of 4 June 2020 amending Annex III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for chlorate in or on certain products. *OJ L 178, 8.6.2020, p. 7–20.*

as regards maximum residue levels for chlorate in or on certain products. Frozen FVH frequently showed chlorate levels higher than those allowed by European legislation in routine controls (EFSA CONTAM Panel, 2015). Consequently, trying to avoid this problem, most of the frozen FBOs stopped using chlorine-based disinfectants and replaced them with PAA or  $H_2O_2$ . The duration of the operational cycle varied among the different case studies. Some operations cycles were about 1–2 h, while in other cases, operational cycles lasted 5 days.

Figure 3 shows the accumulated mass of frozen FVH processed during an operational cycle. Differences were observed among scenarios, with a maximum amount of product being processed of about 1000 tonnes in an operational cycle of about 5 days.

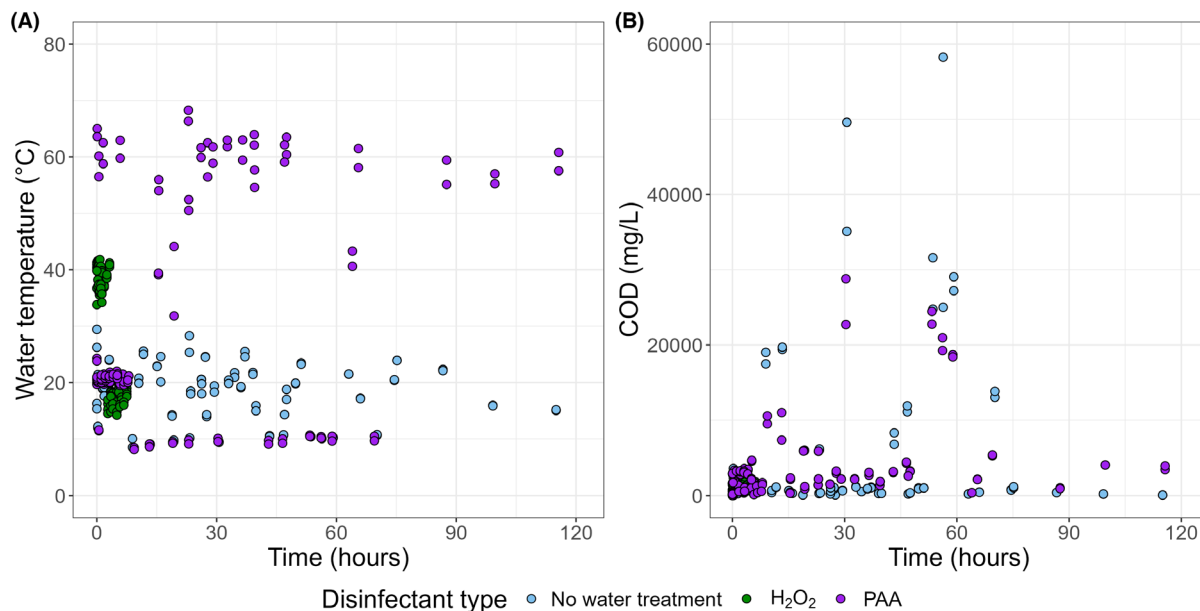


**FIGURE 3** Cumulative mass of the product (tonnes) being processed during each sampling time point of the operational cycle. Source: EFSA outsourced activities (Gil et al., 2025).

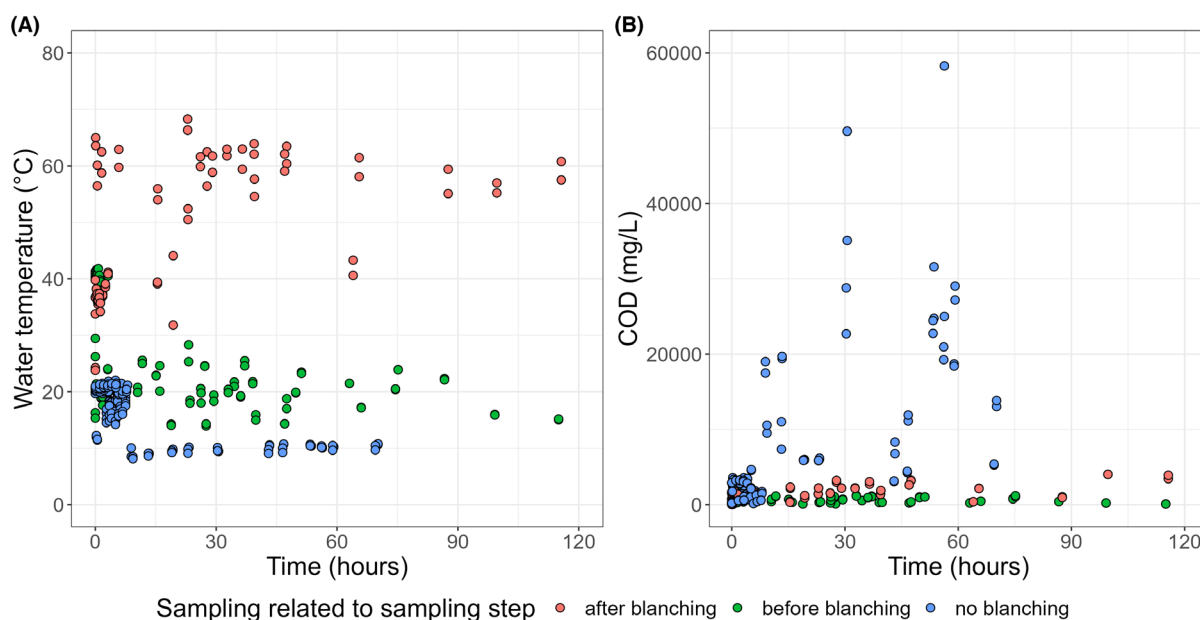
Despite the large mass of product being processed (Figure 3), most of the FBOs only reported a minimum partial refilling of the water tank with unknown volumes of water in this sector. Based on the physico-chemical and microbiological characteristics of the process water during the production, it could be concluded that water replenishment (or refreshment) is not applied as an intervention strategy (EFSA outsourced activities – Gil et al., 2025). FBOs only performed refilling of 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 frozen FVH along the operational cycle in scenarios with no water treatment and in those using hydrogen peroxide and PAA as disinfectants.

For the frozen sector, only a few scenarios ( $n = 3$ ) included the cooling operation after blanching. In order to depict how the blanching step may influence specific characteristics of the process water, data are represented in Figure 5 considering the moment at which sampling took place related to the blanching step (1) no blanching, (2) before blanching and (3) after blanching.



**FIGURE 4** Water temperature (°C) (A) and chemical oxygen demand (COD, mg/L) (B) of the process water applied for frozen FVH during each operational cycle. H<sub>2</sub>O<sub>2</sub>: hydrogen peroxide; PAA: peroxyacetic acid. Source: EFSA outsourced activities (Gil et al., 2025).



**FIGURE 5** Water temperature (°C) (A) and chemical oxygen demand (COD, mg/L) (B) of the process water applied for frozen FVH during each operational cycle. Industrial cases were represented based on the blanching step. Source: EFSA outsourced activities (Gil et al., 2025).

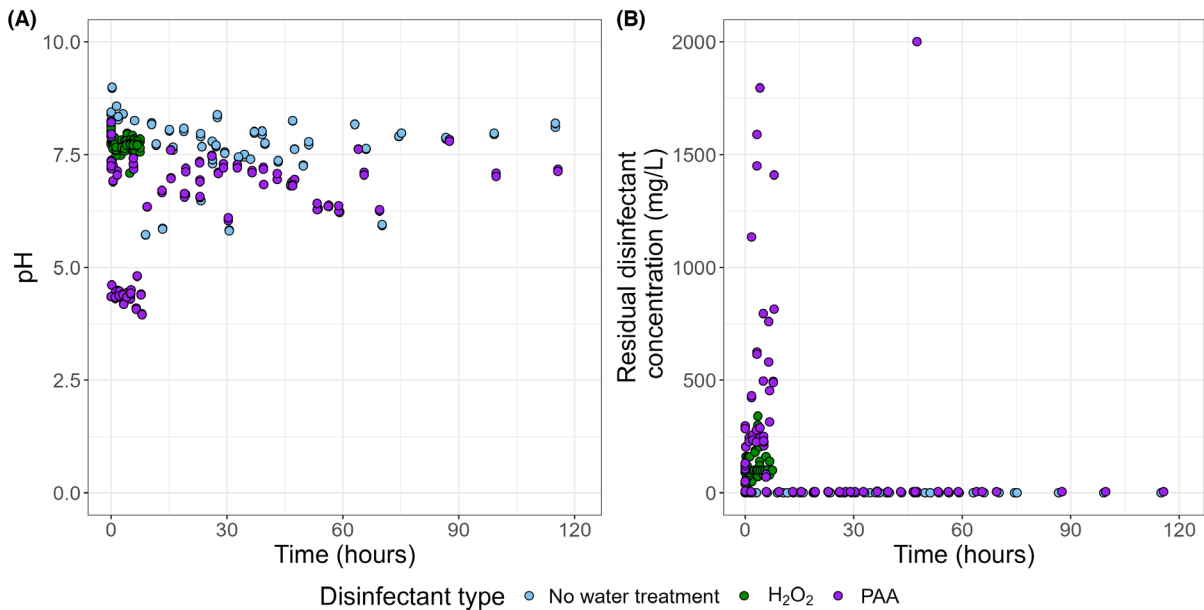
Based on the information retrieved from EFSA's outsourced activities, the process water for the frozen FVH sector is not cooled (Figures 4A, 5A). Cooled conditions are recommended to keep the quality of the product as well as to avoid microbial growth (EFSA BIOHAZ Panel, 2023). For the three scenarios considering cooling after blanching, average temperatures were around 37°C (Scenario ID 54) and almost 60°C (Scenarios ID 58 and ID 59; Figure 5A). During the cooling of blanched FVH, the temperature of the cooling water increases due to the contact with the hot FVH, and thus, cooled conditions will be hard to keep. However, when water is recirculated and reused, cooling the process water is advisable to avoid microbiological growth in water rich in organic matter and used at ambient temperatures. For example, the microbiological load of cooling water in Scenario ID 54 (37°C) is very high, with TBC and TC > 8 log<sub>10</sub> CFU/100 mL (Figure 8A).

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 (from 0 to more than 4500 mg/L) (Figures 4B, 5B). The highest COD concentrations (up to 60,000 mg/L) corresponded to those scenarios where onions are being processed (Scenarios ID 49, ID 55, ID 56 and ID 57) (Appendix B). None of these scenarios corresponded to blanched products, where the COD could be expected to be even higher due to injury of the plant tissue. Excluding the scenarios with onions, most of the cases (75%) showed COD concentrations between 440 and 1700 mg/L. Increases in the COD of the process water can be associated with different factors, including (1) the large mass of product being washed; (2) using refilling only and, therefore, no dilution; (3) the release of nutrients after cutting and blanching; and (4) the type of water disinfection treatment being applied.

For instance, the use of PAA, an organic acid, to maintain the microbiological quality of process water significantly increases the COD of the water (EFSA BIOHAZ Panel, 2023) as it increases the organic load of the process water. Therefore, in most of the cases, those scenarios where PAA is applied showed high COD concentrations compared to when  $H_2O_2$  is applied (e.g. Scenario ID 55). Detailed information related to the industrial data can be found in Gil et al. (2025).

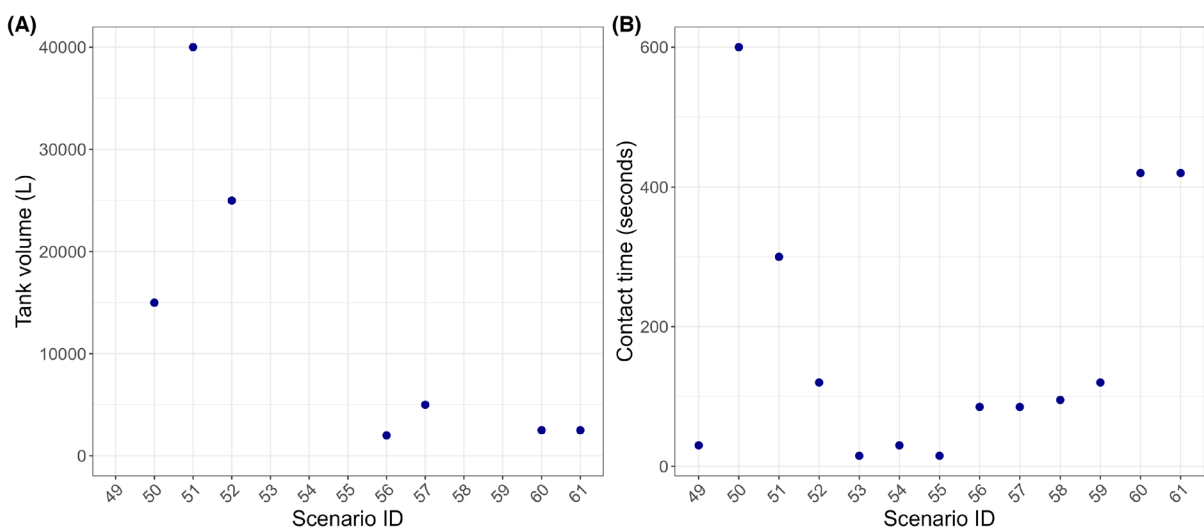
The pH of the process water was, in most of the cases, around 7.5 (Figure 6A). The industrial data included scenarios with and without disinfectant treatment. In most cases (75%), pH values were between 7.8 and 8.3. In the frozen FVH sector, as mentioned before, chlorine-based disinfectants were not used in any of the scenarios, which make the control of pH less critical. The lowest pH values (< 4.5) observed belonged to those scenarios using very high residual concentrations of PAA (between 200 and 1500 mg/L) as water disinfectant (Scenarios ID 60 and ID 61) (Gil et al., 2025).

In those scenarios where water disinfectants (PAA or  $H_2O_2$ ) were used, the residual concentration was very variable (Figure 6B). When PAA was used, residual concentrations were, in general, between 0 and 500 mg/L. One scenario (Scenario ID 60, parsley) presented very high levels of PAA, between 600 and 1600 mg/L. In the case of  $H_2O_2$ , residual concentrations varied from 40 to close to 340 mg/L. Detailed information related to the industrial data can be found in Gil et al. (2025).



**FIGURE 6** pH (A) and residual concentration of disinfectant (mg/L) (B) of the process water applied for frozen FVH during each operational cycle.  $H_2O_2$ : hydrogen peroxide; PAA: peroxyacetic acid. Source: EFSA outsourced activities (Gil et al., 2025).

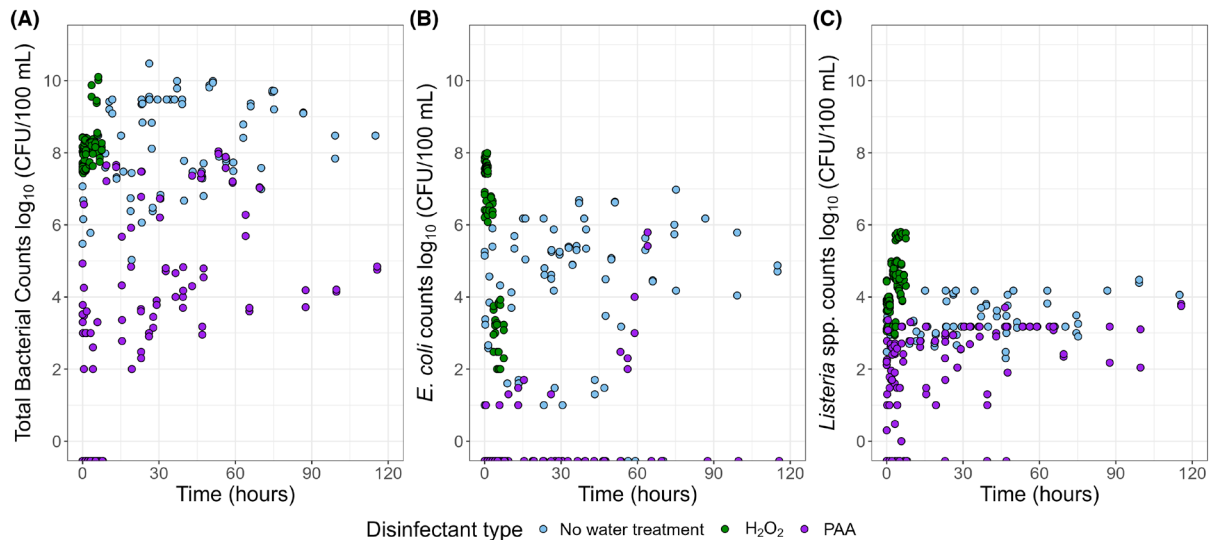
The volumes of water applied were very variable (Figure 7A). The water tanks included in the frozen FVH scenarios had a water volume between 2000 and 40,000 L. The contact times between water and product were also very variable, ranging from 30 s to up to 10 min. However, in most cases, the contact times were below 2 min (Figure 7B). Detailed information related to the industrial data can be found in Gil et al. (2025).



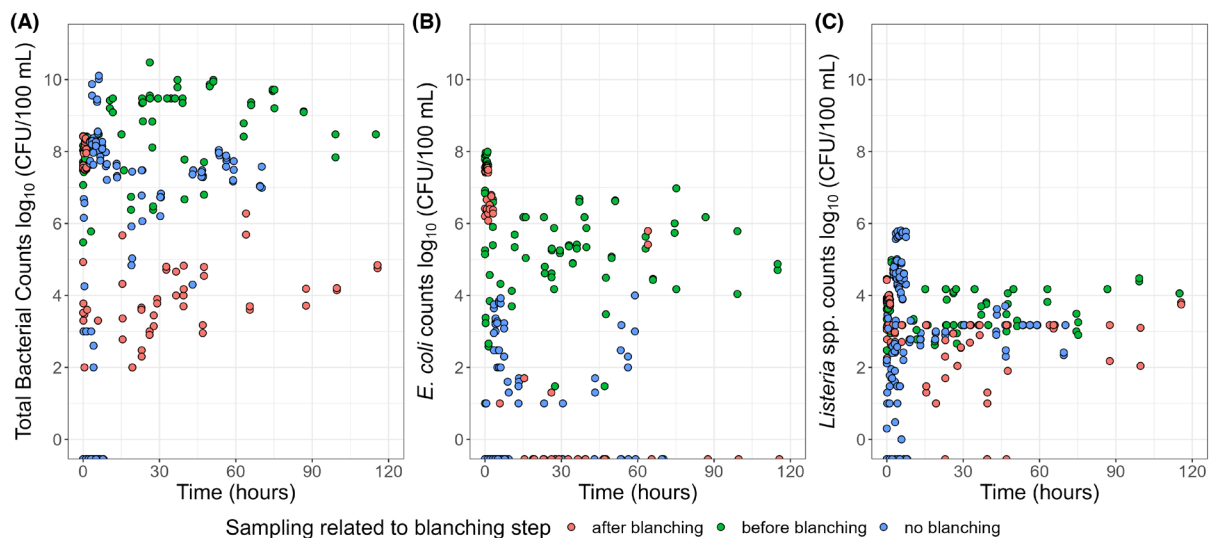
**FIGURE 7** Tank volume water (L) (A) and contact time (seconds) between product and water (B) in the process water samples from the frozen FVH scenarios ID 49 to ID 61 during each operational cycle. For some scenarios (ID 49, 53, 54, 55, 58 and 59), the tank volume is either unknown or not applicable, e.g. when using a water spray instead of immersion in a tank. Source: EFSA outsourced activities (Gil et al., 2025).



The microbial accumulation in the process water during processing and handling operations of the frozen FVH sector is represented in Figures 8 and 9.



**FIGURE 8** Distribution of total bacterial counts (A), *E. coli* (B) and *Listeria* spp. counts (C) (in log<sub>10</sub> (CFU/100 mL)) in process water treated with peroxyacetic acid (PAA), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) or without water treatment in industrial scenarios during each operational cycle of frozen FVH. Bacterial counts for the three microbial groups (A, B and 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.). Source: EFSA outsourced activities (Gil et al., 2025).



**FIGURE 9** Distribution of total bacterial counts (A), *E. coli* (B) and *Listeria* spp. counts (C) (in log<sub>10</sub> CFU/100 mL) in process water sampled after blanching, before blanching or when no blanching was applied in industrial scenarios during each operational cycle of the frozen FVH. Bacterial counts for the three microbial groups (A, B and C) below the respective limit of detection 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.). Results shown in this figure are from samples collected in scenarios where the targeted operation took place before blanching and in other distinct scenarios where the targeted operation took place after blanching and these results are not related to each other. Source: EFSA outsourced activities (Gil et al., 2025).

Levels of TBCs in the process water were very variable among the different scenarios (ranging from 2 up to 10 log<sub>10</sub> CFU/100 mL) (Figure 8A). The highest TBC were observed for those scenarios where no water disinfection treatments or H<sub>2</sub>O<sub>2</sub> were applied. In general, process water from the frozen FVH sector showed the highest levels of TBC among the three monitored industrial sectors, i.e. fresh-whole, fresh-cut and frozen FVH. The lowest TBC (< 4 log<sub>10</sub> CFU/100 mL) were observed in those scenarios where PAA was used. Undetectable levels of TBC were only obtained when very high concentrations of PAA were applied (≥ 200 and up to 1800 mg/L). On the other hand, the use of H<sub>2</sub>O<sub>2</sub> did not avoid the accumulation of high levels of TBC (> 7.4 log<sub>10</sub> CFU/100 mL) in the process water (EFSA outsourced activities – Gil et al., 2025). A similar trend, but with lower levels, was observed for the *E. coli* and the *Listeria* spp. counts (Figure 8B,C). The levels of *E. coli* and *Listeria* spp. in process water that was treated and untreated with H<sub>2</sub>O<sub>2</sub> were very high. For PAA, the residual concentrations seem to have an antimicrobial effect, delaying the accumulation of *E. coli*, but are less effective against *Listeria* spp. Detailed information related to the industrial data can be found in Gil et al. (2025).

It could be expected that microbiological levels of process water for the cooling step, after blanching, would be lower than those for washing before blanching (Figure 9). However, *E. coli* levels after blanching were similar to those before blanching (Figure 9B). In fact, although only a few industrial scenarios focused on the use of water after the blanching step ( $n=3$ ), no considerable differences were observed in the microbial load and COD in the process water when comparing the results of operations that took place before or after blanching (Figures 5B and 9A–C).

The levels of VBNC bacterial cells in process water were determined in two scenarios (ID 56 and ID 57). These two scenarios corresponded to FBOps using  $H_2O_2$  as an intervention strategy (EFSA outsourced activities – Gil et al., 2025). In both cases, high levels ( $>8 \log_{10}$  CFU/100 mL) of VBNC were observed. Similar results were observed in Scenario ID 56 for TC, where levels of VBNC cells were about  $1 \log_{10}$  higher than cultivable cells. In these two scenarios, *E. coli* was also detected. In Scenario ID 56, *E. coli* levels of VBNC cells were about three logs higher than those of cultivable cells. In Scenario ID 57, differences between VBNC and cultivable cells were about 2 logs, always higher for the VBNC. These results showed the low efficacy of the applied intervention strategy and the induction of the VBNC cells. 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 frozen FVH sector are as follows:

- (i) The temperature of the process water is not controlled and is usually at room temperature. Process water of the cooling step after blanching showed the highest temperature (20–60°C).
- (ii) The amount of product that is processed during the operational cycle varies among the scenarios, with the most common cumulative values reported between a few tonnes (2–3) and up to 1000 tonnes.
- (iii) Intervention strategies are limited to the use of water disinfection treatments such as PAA and  $H_2O_2$ . PAA was the most used (5 scenarios). Chlorine-based disinfectants were not used in this sector, which differs from the fresh-whole and fresh-cut FVH sectors.
- (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) A wide variability of water volumes in operation tanks was observed (range: 2000–40,000 L). The most common water source was recycled water.
- (vi) A wide variability of product-to-water contact times was observed among the different operations (range: 0.5–10 min), but in most of the cases, short contact times were observed (e.g. less than 2 min).
- (vii) The highest microbiological load of the process waters for TBC, TC, *E. coli* and *Listeria* spp. were found when compared with the other FVH sectors (fresh-whole and fresh-cut FVH). The water management practices applied in the frozen sector are not efficient in maintaining the microbiological quality of the process water. Only the use of very high concentrations of PAA (200–1600 mg/L) managed to avoid the accumulation of microorganisms in the process water.
- (viii) Seasonal work: Some commodities are only available for a few weeks during the year, and the production is concentrated during these weeks (e.g. for products like broccoli, peas and spinach).

### 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 main pathogens based on an EFSA FoNAO opinion (EFSA, 2013), reported European outbreaks between 2014 and 2020 and a literature review (publications published between 2010 and 15-2-2022). Regarding the main food–pathogen combinations, the far most common combinations were outbreaks involving enteric viruses (NoV and HAV) on frozen berries. Besides that, frozen corn was the vehicle for a multi-country listeriosis outbreak (EFSA BIOHAZ Panel, 2023). There is a lack of evidence of *Salmonella* and STEC outbreaks caused by the consumption of frozen FVH. However, *Salmonella* spp. and STEC can colonise production environments, but, as stated in (EFSA BIOHAZ Panel, 2023), the most likely introduction route is attributed to the raw produce (EFSA BIOHAZ Panel, 2024). Due to the potential to colonise a wide range of products and the impact in terms of morbidity and mortality, these bacterial pathogens could still be considered in the hazard analysis (EFSA BIOHAZ Panel, 2023).

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 13 different scenarios (Gil et al., 2025). All pathogen findings are listed in Appendix B. *Salmonella* spp. was detected on several occasions in seven different scenarios, namely in water used for washing and cooling spinach and for transporting and cooling onions. In most of these scenarios, recycled water with no disinfection was used. Further, *L. monocytogenes* could be detected in several of these scenarios, whereas there was only one positive finding of STEC (washing of spinach) (Appendix C).

Norovirus and *Cryptosporidium* spp. were only analysed on a single sampling time point (i.e. the last out of the six defined sampling time points) for each scenario. Whereas norovirus genomes were detected in several process water samples, *Cryptosporidium* spp. was never detected (Appendix C). The detection method used for norovirus GI after the addition of PMAxx indicates intact virions but not necessarily their infectivity.

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 for the frozen processing industry are:

a) **Contaminated water source.** Different post-harvest and handling operations (transport, washing, blanching, post-blanching) used recycled water without the implementation of any physical or chemical intervention strategy in between uses. Recycled water was the source in several scenarios with multiple findings of *L. monocytogenes* and *Salmonella* spp., even at early sampling time points of the operational cycle, when no disinfection was applied (Appendix C), potentially leading to cross-contamination of the final product.

b) **Use of the same water to wash large volumes of product during the operational cycle without a well-managed intervention strategy.** In several industrial scenarios, it was observed that the same water was used in a single operation without the application of a well-managed physical and/or chemical intervention strategy during the whole operational cycle. The use of the same water in contact with large volumes of product for long processing operations without water replenishment will build up contamination in the water and may contribute to batch-to-batch contamination. All pathogenic bacteria findings were observed in scenarios with longer durations (> 2400 min) before complete removal of the water used in the tank, whereas *Salmonella* spp. or *L. monocytogenes* could not be detected in scenarios with shorter operational cycles (ID 56, ID 57, ID 60 and ID 61) (EFSA outsourced activities – Gil et al., 2025).

c) **Incomplete removal of the water used in the water tank.** At several occasions pathogens were detected at sampling time point 1 despite limited, or as in Scenario ID 50, no product had been introduced to the washing tank (Appendix C) indicating incomplete removal of water and/or poor cleaning and disinfection in between operations.

d) **Improper cooling process water after blanching.** In the cooling process, the temperature of the water is increased due to the temperature of the incoming product after blanching, and organic material is released, enabling bacterial growth in post-blanching water. It could be expected that the cooling tank after blanching could have low levels of microorganisms due to the heat treatment during blanching. However, high levels of microbial indicators were observed from the beginning to the end of the operational cycles in cooling after blanching (i.e. Scenarios ID 54, ID 58 and ID 59) (EFSA outsourced activities – Gil et al., 2025).

### 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 represent a unique and comprehensive database of industrial scenarios, offering valuable insights into the contamination dynamics of process water across various operations in the frozen 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 operational 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 frozen FVH, the microbiological data were analysed in three steps:

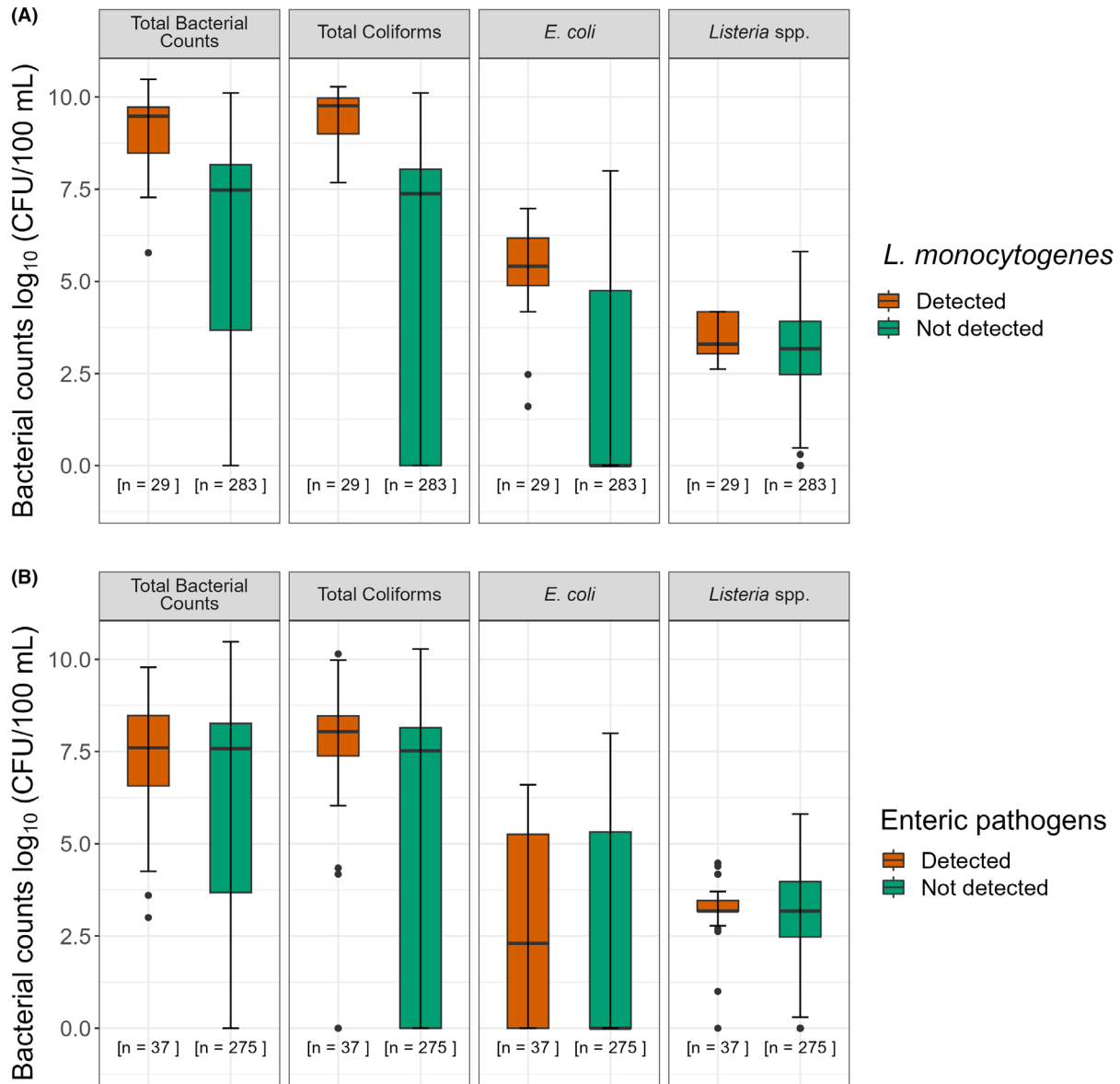
##### 3.3.1.1 | Exploratory analysis of the results by individual samples

Bacterial pathogens were detected in 60 out of 312 water samples analysed, belonging to 7 out of 13 scenarios. *L. monocytogenes* was detected in 29 water samples, *Salmonella* spp. was detected in 36 water samples, while STEC was detected in only one water sample. Six samples were positive for both *Salmonella* spp. and *L. monocytogenes*. Given these results, data associated with the detection of *L. monocytogenes* and the detection of enteric pathogens (agglutination-confirmed *Salmonella* and/or PCR-confirmed STEC)<sup>8</sup> were used to assess the suitability of TBC, TC, *E. coli* and *Listeria* spp. as potential microbial indicators of pathogen contamination.

In Figure 10, the counts of TBC, TC, *E. coli* and *Listeria* spp. in log<sub>10</sub> CFU/100 mL for individual samples taken are displayed as a combined box plot for samples in which *L. monocytogenes* or enteric pathogens (agglutination-confirmed *Salmonella* and/or PCR-confirmed STEC)<sup>9</sup> were detected and not detected (shown in different colours).

<sup>8</sup>Thirty-six out of 37 detected enteric pathogens were *Salmonella*-positive samples, with all 36 confirmed by a Latex agglutination test.

<sup>9</sup>Thirty-six out of 37 detected enteric pathogens were *Salmonella*-positive samples, with all 36 confirmed by a Latex agglutination test.



**FIGURE 10** Box plot of levels (log CFU/100 mL) of total bacterial counts (TBC), total coliforms (TC), *E. coli* and *Listeria spp.* in individual samples of process water taken at different processing operations of frozen FVH scenarios in which the pathogen *L. monocytogenes* (A) and enteric pathogens (agglutination-confirmed *Salmonella* and/or PCR-confirmed STEC)<sup>10</sup> (B) was 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<sub>10</sub> 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 *L. monocytogenes* were generally higher than the levels found in samples in which this pathogen was not detected. More specifically:

- TBC were above 10<sup>6</sup> CFU/100 mL in 99.5% of the samples positive for *L. monocytogenes* and in 64% of the samples negative for *L. monocytogenes*.
- TBC were above 10<sup>5</sup> CFU/100 mL in 100% of the samples positive for *L. monocytogenes* and in 66% of the samples negative for *L. monocytogenes*.
- TBC were above 10<sup>4</sup> CFU/100 mL in 100% of the samples positive for *L. monocytogenes* and in 73% of the samples negative for *L. monocytogenes*.

The levels of **TC** in samples positive for *L. monocytogenes* were generally higher than the levels found in samples in which the pathogen was not detected. More specifically:

<sup>10</sup>Thirty-six out of 37 detected enteric pathogens were *Salmonella*-positive samples, with all 36 confirmed by a Latex agglutination test.

- TC were above  $10^4$  CFU/100 mL in 100% of the samples positive for *L. monocytogenes*, while such a level of TC was only observed in 71% of the samples negative for the pathogen.
- TC were above  $10^3$  CFU/100 mL in 97% of the samples positive for *L. monocytogenes*, while such a level of TC was observed in 65% of the samples negative for the pathogen.
- TC were above  $10^2$  CFU/100 mL in 93% of the samples positive for *L. monocytogenes*, while such a level of TC was observed in 63% of the samples negative for the pathogen.

To sum-up, high levels of **TBC** and **TC** were found in process water both in samples which were positive and negative for *L. monocytogenes* during the whole operational cycle, i.e. from the first sampling time point until the last sampling time point. Therefore, the use of TBC and TC as indicators for the detection of *L. monocytogenes* is not appropriate based on the data set collected through EFSA's outsourced activities.

The levels of *E. coli* were generally higher in samples positive for *L. monocytogenes* compared with water samples in which the pathogen was not detected. When looking at the distribution of the levels of *E. coli*:

- *E. coli* was above 100 CFU/100 mL in 98% of the samples positive for *L. monocytogenes*, while such a level of the indicator was only observed in 40% of the samples negative for the pathogen.
- *E. coli* was above 10 CFU/100 mL in 100% of the samples positive for *L. monocytogenes*, while such a level of the indicator was only observed in 47% of the samples negative for the pathogen.
- *E. coli* was above 1 CFU/100 mL in 100% of the samples positive for *L. monocytogenes*, while such a level of the indicator was only observed in 48% of the samples negative for the pathogen.

The levels of *Listeria spp.* in water samples positive for *L. monocytogenes* were within the range of the *Listeria spp.* levels found in samples in which the pathogen was not detected. Therefore, the data collected through EFSA's outsourced activities did not indicate *Listeria spp.* as a potential microbial indicator of the detection of *L. monocytogenes*.

### 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 13 different scenarios, at least one of the investigated pathogens was detected in more than one sampling time point of each of the two sampling visits (corresponding to Scenarios ID 49, ID 50, ID 51, ID 52, ID 55 and ID 59) and one sample in one of the sampling visits of Scenario ID 58.

*L. monocytogenes* was detected in Scenarios ID 49, ID 50, ID 51 (two visits – ID 52 and ID 55), *Salmonella spp.* was detected in Scenarios ID 49 (two visits), ID 50 (two visits), ID 51, ID 52 (two visits), ID 55 (two visits), ID 58 and ID 59 (two visits). Some samples showed positive results for both pathogens in the following scenarios: ID 49, ID 50, ID 51, ID 52 and ID 55 (Appendix C).

STEC was only detected in the washing of spinach using recycled water (scenario ID 52), in which the other pathogens were also detected combined with a high COD and turbidity (Gil et al., 2025).

The relationship between the occurrence of potential microbial indicators above a given threshold and the detection of *L. monocytogenes* or enteric pathogens (agglutination-confirmed *Salmonella* and/or PCR-confirmed STEC)<sup>11</sup> within each sampling visit was assessed. OR,<sup>12</sup> its 95% CI and the statistical significance were calculated for three different thresholds for each potential indicator (Appendix D), both for *L. monocytogenes* (Table D1) and enteric pathogens (Table D2).

Based on the obtained results, no statistically significant OR was obtained (as confirmed by the 95% CI) except for *E. coli* counts and enteric pathogens, with OR= 11 (95% CI: 1.1–109.7,  $p < 0.05$ ) at the threshold levels of 1 and 10 CFU/100 mL, reinforcing the lack of relationship between the considered type and levels of most of the microbial indicator group and the detection of *L. monocytogenes* in process water.

### 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 in this opinion (frozen FVH) considered only cases of *Salmonella spp.* detection, whereas previous analyses (boxplots and OR shown above) merged the detection of *Salmonella spp.* and STEC as enteric pathogens.<sup>13</sup> This difference is considered negligible, seen in very few cases of STEC detection. Annex B provides a detailed description of the modelling and its outputs. Model 1 suggested that the odds of detection of *Salmonella* would increase

<sup>11</sup>Thirty-six out of 37 detected enteric pathogens were *Salmonella*-positive samples, with all 36 confirmed by a Latex agglutination test.

<sup>12</sup>Odds measure how likely the detection of the pathogen, and is calculated when the indicator is below and above the given threshold.

<sup>13</sup>Thirty-six out of 37 detected enteric pathogens were *Salmonella*-positive samples, with all 36 confirmed by a latex agglutination test.

by a factor of 2.21 for each  $\log_{10}$ -unit increase of the concentration of *E. coli*. The 95% CI shows some uncertainty in the estimate, indicating that the effect could vary from 1.26 to 3.88.

The logistic model 2 suggested that the odds of detection of *Salmonella* in process water in frozen FVH sector would increase by a factor of 2.32 (95% CI 1.40–3.87) for each  $\log_{10}$ -unit increase of the concentration of *E. coli*. Also, in this case, there are uncertainties in the estimates, as shown by the 95% CI. Model 2 also showed a decrease in the odds of detection of *Salmonella* spp. for each  $\log_{10}$ -unit increase of *Listeria* spp. in process water in frozen FVH sector. However, this result has no biological justification.

There are some additional uncertainties affecting the results. Both models showed singularity issues triggered by two random effects having an estimate of zero or close to zero. Moreover, the parameter estimates were not robust but varied depending on the variables included in the model (*E. coli* and TBC in Model 1, *E. coli* and *Listeria* spp. in Model 2), indicating a degree of redundancy in the ability of the microbial indicators to explain the odds of *Salmonella* spp. detection. The only outcome that appears consistently in the two models is the relevant effect of the 'scenario' in explaining the variability of the odds of detection of *Salmonella* spp.

For *L. monocytogenes*, results of the two better fitting models (AIC 117 and 111, respectively, for Model 1\_1 and Model 2\_1) show no statistically significant effects of the levels of potential microbial indicators on the odds of detecting *L. monocytogenes*, after accounting for the hierarchical structure in the data (random effects) and controlling for the effect of the analytical methods used to confirm presumptive *L. monocytogenes*. A large sampling uncertainty characterises all the estimates as reflected by the large standard error and size of the confidence intervals, not allowing firm conclusions to be drawn based on the model. From the comparison between models with and without the analytical method as a fixed effect, it is evident that the latter plays an important role in predicting the odds of detecting *L. monocytogenes*. However, purely qualitative considerations can be made for this factor. Findings for Models 1\_1 and 2\_1 show that the estimate for the PCR method was lower than that for the carbohydrate fermentation-based method. However, this result must be interpreted with caution, considering the large uncertainties in the estimates and the correlation between the method used and the food sectors. The estimates of the random effects are all very large, particularly the one related to the sampling time point. The factor 'scenario' is less prominent in this case but still able to explain part of the variability.

The only conclusion that is consistent among all the 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. In particular, the 'scenario' is associated with the largest variability among random effects when predicting the detection of *Salmonella* spp. When dealing with *L. monocytogenes*, the variability explained by the 'sampling time points' is the largest.

The difference between the two pathogens is probably due to the setting of the multivariable model that estimates the random effects based on the data available for the food sectors all together. In the case of *Salmonella* spp., it was detected in the process water of fresh-cut and frozen FVH but not in process water of fresh-whole FVH, whereas *L. monocytogenes* was detected in the process water of fresh-whole FVH and the frozen FVH sectors but not in fresh-cut FVH. In the fresh-cut FVH sector the operational cycle is usually shorter (ranging from 5 to 15 h) compared to fresh-whole FVH (up to 6 weeks) and frozen FVH (ranging from 8 to 120 h). The greater variability at the level of the 'sampling time point' for *L. monocytogenes* probably stemmed from the sectors considered in the data reflecting overall a longer operational cycle duration as compared to the sectors included in the analysis of *Salmonella* spp. for which variability was larger at the level of the 'scenario'.

It is worth noting that the 'scenario' combines several production 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 these potential microbial indicators for verification purposes within the water management system should be validated under the specific operational conditions of each FBO as described in Section 3.5, particularly when *Salmonella* is the pathogen of concern.

### 3.3.2 | Relationship between microbiological and physico-chemical parameters

Despite comprehensive statistical analysis, including techniques such as PCA, the data set for frozen 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 variability in most of the physico-chemical parameters (e.g. pH) and, on several occasions, the microbial loads (e.g. *E. coli*) fall below detectable levels or are randomly distributed.

Frozen FVH scenarios included in EFSA's outsourced activities did not include chlorine-based disinfectants but only  $H_2O_2$  and PAA. However, the results did not allow the identification of a reasonable target residual concentration activity capable of controlling the microbiological quality of the process water. For instance, despite the high residual concentration of  $H_2O_2$  (180–240 mg/L) in several scenarios (ID 53, ID 54, ID 55, ID 57), high counts of the microbial indicators (TBC, TC, *E. coli* and *Listeria* spp.) were observed. The low residual concentration of PAA (5 mg/L) in Scenarios ID 55, ID 58 and ID

59 did not allow for control of the microbiological quality of the water (pathogens were detected). On the other hand, the low microbiological levels found in process water of Scenarios ID 60 and ID 61 were associated with a very high residual concentration of PAA (up to 1790 mg/L), far above the recommended target (30–80 mg/L, EFSA BIOHAZ Panel, 2023).

Despite the lack of statistical association, it is worth mentioning that within the scenarios using PAA disinfection, *E. coli* was counted only in samples with a residual concentration below 5 mg/L, irrespective of the water source (municipal tap water, surface water, or well water). *Listeria* spp. was detected even in process water with higher residual PAA concentrations (up to 2000 mg/L). Within the scenarios using H<sub>2</sub>O<sub>2</sub> disinfection, *E. coli* was counted only in samples with a residual concentration below 160 mg/L, irrespective of the water source (municipal tap water, surface water, or well water). *Listeria* spp. was always detected in treated process water at higher residual H<sub>2</sub>O<sub>2</sub> concentrations (up to 340 mg/L).

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 13 scenarios available in the data set collected through EFSA's outsourced activities for frozen FVH (Section 3.1.2) were systematically screened, and hazardous events were identified. In this section, these hazardous events (Section 3.2) are linked to specific preventive measures where additional attention is requested for this sector. In Table 2, specific highlights for the frozen 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, seasonal work) and routine measures (i.e. in the daily routine operation of industry) in the frame of specific PRPs. A full description of generic pre-requisite measures (13 PRPs) can be found in the EC Commission Notice 2022<sup>14</sup> and PRPs specific for water management, distribution and storage systems in EFSA BIOHAZ Panel (2023).

<sup>14</sup>Commission notice on the implementation of food safety management systems covering Good Hygiene Practices and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses (2022/C 355/01). 16.9.2022, p. 1–58.

**TABLE 2** Specific points of attention in the preventive measures, as defined in the EC Commission Notice 2022/C 355/01<sup>15</sup> 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 frozen FVH, based on the identified hazardous events (Section 3.2).

Preventive measure	Specific attention to preventive measures for frozen FVH		
	PRESTART MEASURES	RESTART MEASURES	ROUTINE MEASURES
<b>PRE-REQUISITE (EC Commission Notice 2022/C 355/01)</b>			
<b>PRP 1: Infrastructure and fit-for-purpose building and equipment</b>	<p>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.</p> <p>Segregation in a cleaner area (higher hygiene level) of post-blanching activities is recommended to avoid cross-contamination.</p> <p>Set up a well-designed and managed water disinfection system as an intervention measure (installation of pumps, dosing systems, sensors, valves, etc.)</p>	<p>Every time the processing line is restarted, the infrastructure and equipment should be revised.</p>	<p>A monthly visual check based on a checklist of infrastructure (hygiene and condition).</p> <p>Monitoring the proper working of the water management system (see Section 3.6)</p>
<b>PRP 2: Cleaning and disinfection</b>	<p>The equipment used for the cleaning and disinfection of the water, as well as the water distribution and storage systems, need to be revised before activities are started.</p>	<p>In case of restart after a prolonged stand-still (as in the case of seasonal activities), cleaning and disinfection need to be conducted to prevent microbial contamination of water and biofilm formation.</p>	<p>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.</p> <p>Dedicated time surely needs to be foreseen when continuous and/or long-time runs occur in the processing of frozen FVH.</p> <ul style="list-style-type: none"> <li>– Spot visual checks</li> <li>– Daily visual checks</li> </ul> <p>Perform microbiological environmental testing.</p> <p>In seasonal production peaks, a routine cleaning and disinfection regimen should also be included.</p>

<sup>15</sup>Commission notice on the implementation of food safety management systems covering Good Hygiene Practices and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses (2022/C 355/01). 16.9.2022, p. 1–58.



TABLE 2 (Continued)

Preventive measure	Specific attention to preventive measures for frozen FVH		
	PRESTART MEASURES	RESTART MEASURES	ROUTINE MEASURES
<p><b>PRE-REQUISITE (EC Commission Notice 2022/C 355/01)</b></p> <p><b>PRP 4: Technical maintenance and calibration</b></p>	<p>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).</p>	<p>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 seasonal work.</p>	<p>Routine inspection and maintenance of equipment are needed to prevent contamination of fit-for-purpose water with potentially contaminated water.</p> <p>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.</p> <p>Monitoring equipment needs to be calibrated, e.g. thermometers, water flow meters and disinfection dosing systems, so that proper use can be guaranteed.</p> <p>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 for spraying water on frozen product (glazing).</p> <p>In seasonal production peaks, a preventive technical maintenance regimen should also be included.</p>
<p><b>PRP 8: Water and air control</b></p>	<p>Cooling post-harvest water to reduce microbial growth. Post-blanching water temperature should be cold enough to avoid growth in this phase of the production.</p> <p>Frequency of complete removal of water in tanks/process lines needs to be established.</p>	<p>Cooling post-harvest water to reduce microbial growth.</p> <p>Every time the processing line is restarted, the water in the process lines should be completely removed and new fresh water inserted.</p>	<p>Cooling post-harvest water to reduce microbial growth.</p> <p>The microbiological quality of water applied for crystallisation/glazing should also be considered to avoid potential product contamination (potable water).</p> <p>Routine inspection of the frequency of complete removal of the water in the processing lines is needed to avoid prolonged use of the process water (and, therefore, avoid the use of the same water to wash large volumes of product over different operational cycles).</p>
<p><b>PRP 9: Personnel (hygiene, health status)</b></p>	<p>Appropriated training for operators and team leaders is due to the nature of automated and seasonal production systems, manual labour (e.g. manual sorting), and the creation of a positive food safety culture and awareness of hygiene and microbiological food safety.</p>		<p>Retake the training with a certain frequency.</p>

**TABLE 2** (Continued)

Preventive measure	Specific attention to preventive measures for frozen FVH		
	PRESTART MEASURES	RESTART MEASURES	ROUTINE MEASURES
<b>PRE-REQUISITE (EC Commission Notice 2022/C 355/01)</b>  <b>PRP 12: Working methodology</b>	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)  <b>SOP includes logistic management:</b> 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, potatoes).  <b>SOP include reusing water:</b> 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 first washing of FVH.  <b>SOP includes the use of disinfection techniques:</b> clear explanation of the dose, monitoring, etc., and what to do in case the disinfection is not working appropriately (e.g., actions to take to process and product).

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

Intervention measures include 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 frozen FVH. In the Part 1 Opinion, two main intervention measures were identified: Water replenishment and water disinfection treatments (EFSA BIOHAZ Panel, 2023). However, based on EFSA's outsourced activities, only chemical water disinfection treatments (i.e. PAA and H<sub>2</sub>O<sub>2</sub>) were applied by some FBOs from the frozen FVH sector (Table 3). In this case, as presented in Table 4, four Scenario Groups (SG) were identified, which cover the above-mentioned intervention measures. The SGs considering the application of a water disinfection treatment are based on the application of chlorine-based disinfectants (e.g. sodium and calcium hypochlorite), as it was deemed suitable to illustrate the impact of disinfectant-based intervention. 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 3** Types of intervention measures considered in this scientific opinion and the number of scenarios found in the frozen FVH industrial data included in EFSA's outsourced activities ( $n=13$  scenarios from two different European countries) (Source: EFSA outsourced activities (Gil et al., 2025)).

Intervention measures	Number of scenarios from EFSA's outsourced activities
None	4
Water disinfection (WD)	9
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 other situations that were not found among the industrial cases, representing an adequate water safety management (e.g. SG 2.2 and SG 4; Table 4), a mathematical model was used to simulate hypothetical scenarios representative of the frozen 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>).

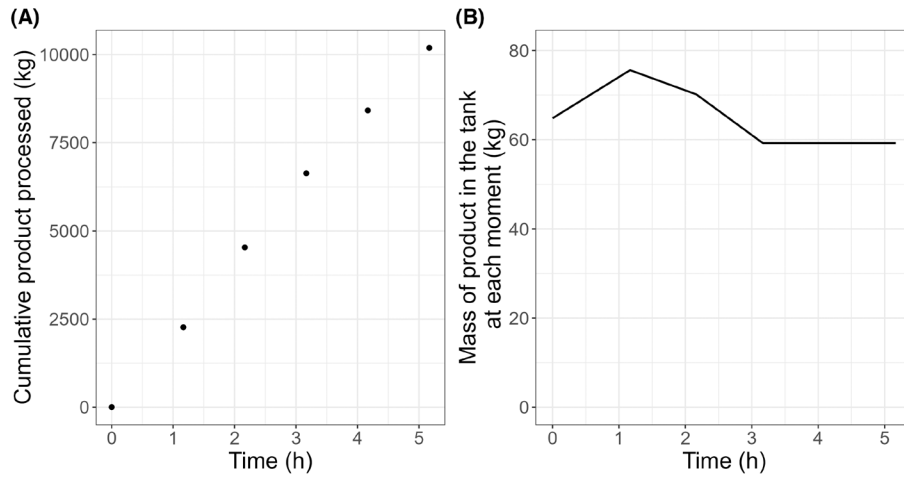
To compare the outputs of the model when different intervention measures are applied, all the simulations refer to a (hypothetical) frozen product (i.e. frozen chives), based on scenario ID 61, sampling visit 1 (Gil et al., 2025), with an operational cycle of 5 h, running under the same operational conditions (e.g. volume of water 2500 L and total amount of product 10,192 kg) as shown in Figures 11, 12 and Table 4. 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).

Tables E.1 and E.2 in Appendix E summarise all the model parameters and input data used for variables in the model simulations of all the different scenario groups in the frozen FVH sector.

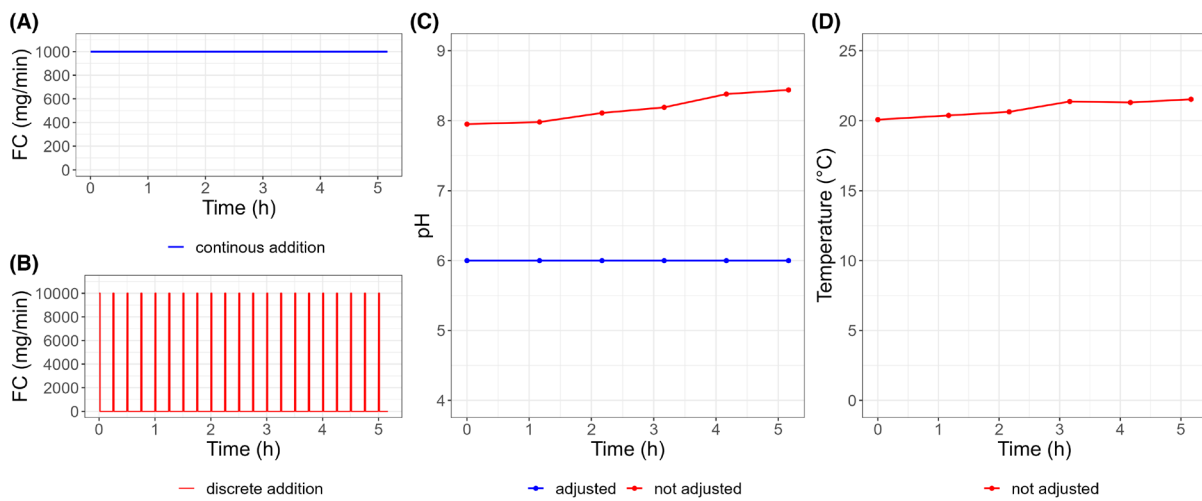
**TABLE 4** Conditions<sup>a</sup> of application of the simulated intervention measures and physico-chemical parameters (FC, pH and temperature) used as input values for model simulations of all the scenario groups representative of the frozen FVH sector.

Scenario group	Intervention measures			Physico-chemical parameters	
	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: 8.0–8.5	Not adjusted: 20.0–21.5
Scenario Group 2.1	20	Discrete: 10,000 mg every 15 min	Water refilling: 0.5	Not adjusted: 8.0–8.5	Not adjusted: 20.0–21.5
Scenario Group 2.2	20	Continuous: 1000 mg/min	Water refilling: 0.5	Adjusted: 6	Adjusted: 5
Scenario Group 3	0	None	Water replenishment: 25	Not adjusted: 8.0–8.5	Not adjusted: 20.0–21.5
Scenario Group 4	20	Continuous: 1000 mg/min	Water replenishment: 25	Adjusted: 6	Adjusted: 5

<sup>a</sup>Based on scenario ID 61, sampling visit 1 EFSA's outsourced activities (Gil et al., 2025), with an operational cycle of 5 h, running under the same operational conditions (e.g. volume of water 2500 L and total amount of product 10,192 kg, contact time 2 min).

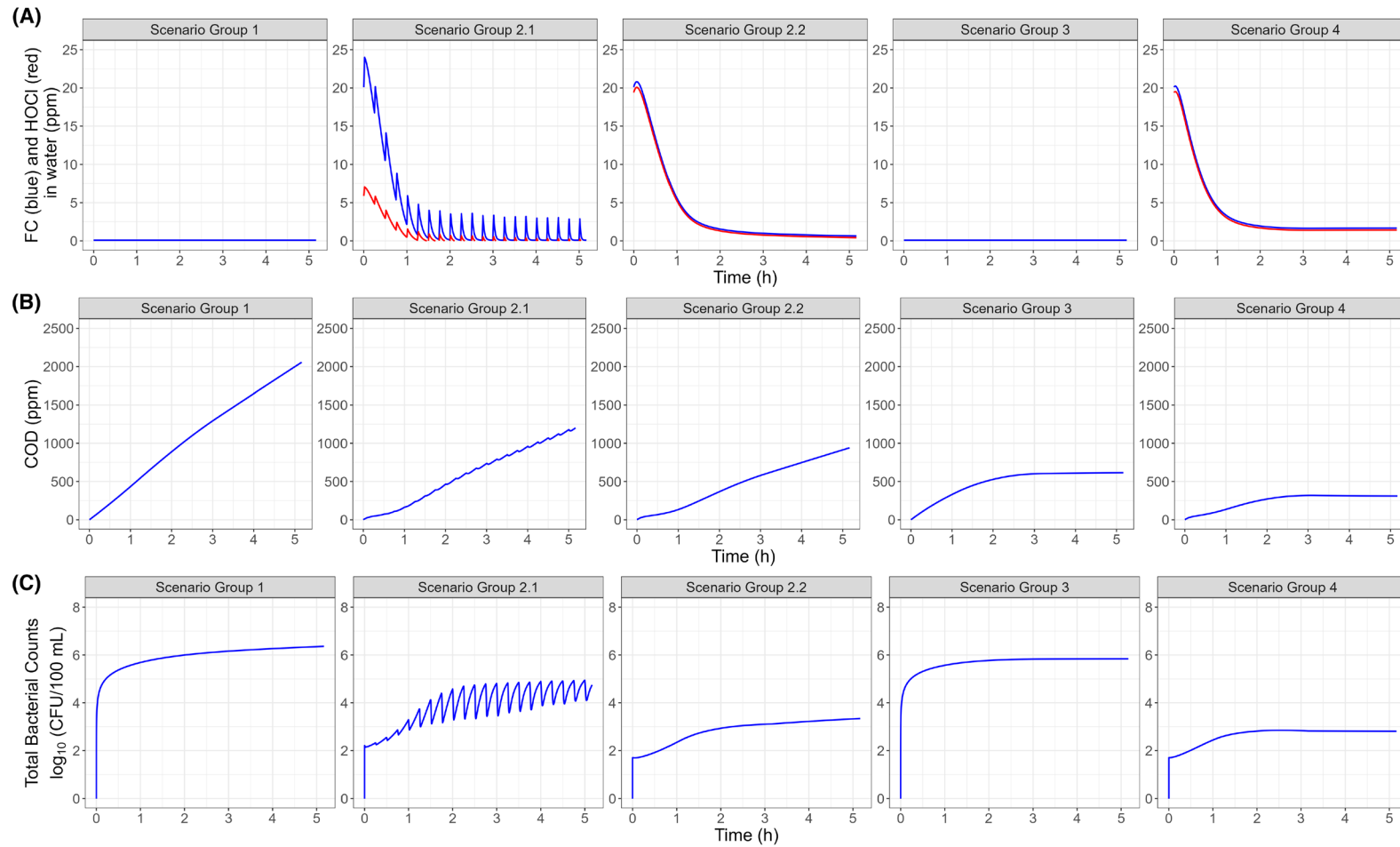


**FIGURE 11** Cumulative mass of the product being processed (A) and mass of the product in the tank at each moment (B) during the 5-h operational cycle used in the simulations of the scenario groups selected as representative of the frozen FVH sector.



**FIGURE 12** Changes in physico-chemical parameters FC dosing (A: Continuous addition, B discrete addition), pH (C) and temperature (D) used as model inputs for the simulation of the scenario groups of the frozen 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 13 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 13 refers to one type of SG.



**FIGURE 13** 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 frozen FVH sector (see Table 4 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 13A), and only water refilling at a constant rate (0.5 L/min) has been considered (Table 5). The model simulations clearly show that in the absence of any intervention strategy, the COD (Figure 13B) and the microbial load (Figure 13C) of the initially clean water constantly increase throughout the operational cycle, reaching levels up to ca.  $6 \log_{10}$  CFU/100 mL and 2000 mg/L, respectively (Figure 13). 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-scenarios 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. Water disinfection treatment was applied without properly adjusting the FC 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, as well as hypochlorous acid, is minimal during the operational cycle (Figure 13A). As a result, the organic matter calculated as COD goes up to 2000 mg/L (Figure 13B), and microorganisms increase close to  $6 \log_{10}$  CFU/100 mL (Figure 13C)
- **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, i.e. the fraction of FC with the highest antimicrobial efficacy) was present above 5 mg/L during the first hour of the operational cycle (Figure 13A). Compared with SG 1 (no intervention), the increase of the levels of COD never exceeded 1000 mg/L (Figure 13B), and the accumulation of microorganisms was controlled below  $3.5 \log_{10}$  CFU/100 mL throughout the operational cycle (Figure 13C). Although the same flow rate of chlorine dosing was maintained during the operational cycle, the chlorine demand increased due to the increase of organic matter with the addition of products being processed (as shown by COD). 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 after 2h of the operational cycle (Figure 13).

**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 13A). The water replenishment is defined in the model by increasing the addition of water from 0.5 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 levels when compared to SGs 1 and 2, mostly due to the dilution effect of the high addition of water (Figure 13B). 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 13C).

**Scenario Group 4** represents the situation where both water disinfection and water replenishment (25 L/min) interventions are applied to maintain the microbiological quality of process water. 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 11). 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 a significant impact, avoiding the accumulation of organic matter in the process water; thus, COD remains below 300 mg/L (Figure 13B). As a consequence, compared with SG 2.2, despite the initial higher dilution of the FC residual concentration, it is kept at a slightly higher level since the oxidation of FC by organic matter is lower (Figure 13A). Therefore, the combination of water disinfection (to control the accumulation of microbial load) and water replenishment (to dilute organic matter, 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 13C).

### 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 frozen 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 frozen 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 in the verification of 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 frozen FVH production**

For the specific processing operations applied in frozen FVH, the typical characteristics of the water used need to be set, based on the flow chart in [Figure 1](#) defined for a 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.
- Blanching and cooling after blanching: specific steps for some frozen FVH production, heating of water, recirculation of water, refill to compensate the loss of water and/or use of replenishment.
- Glazing is a specific process step for some frozen FVH production where water is sprayed over the frozen FVH to avoid freeze-drying during prolonged storage in deep frozen conditions. In this case, the water is part of the final product.

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

For each of the specific processing operations applied in frozen 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, type of intervention measure (if applicable), blanching conditions (contact time/temperature), cooling conditions (contact time/temperature).
- (2) Product-related factors, both
  - Fixed factors that remain constant in every processed batch, such as type(s) of commodity, product feed rate, etc., 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 produce 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 certain time frame), the product-to-water ratio, water temperature (particularly in the cooling after blanching), etc.
  - Dynamic factors that 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 that 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 real 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, and 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 predictive 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) that 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 the application of interventions or their combinations on the resulting process water quality using mathematical models developed 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](#)). A 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 in 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 TBC as the microbial indicator 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 1–4 (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.

The target quality of water will depend on the non-RTE or RTE status of the frozen FVH (EFSA BIOHAZ Panel, 2023; PROFEL, 2020). In case the FBOp wants to commercialise frozen FVH as RTE, they need to govern their activities accordingly. A higher quality of water or even potable water quality is needed for process water quality (e.g. water applied for the glazing of the produce).

EFSA's outsourced activities were not aimed at performing a validation study, and neither TBC, TC, nor *Listeria* spp. levels could be clearly associated with the detection of pathogens in process water for frozen FVH. However, it is noteworthy to highlight that in EFSA's outsourced activities, only a few ( $n=2$ ) of the scenarios of the frozen FVH sector showed levels of TBC, TC and *E. coli* below the detection limit in most of the samples, and *Listeria* spp. below 100 CFU/100 mL in 80% of the samples. For the other scenarios, high levels of indicator microorganisms were enumerated from the beginning of the operation (sampling time zero), making it difficult to establish potential microbial indicators and their thresholds.

On the other hand, *E. coli* levels were higher in process water positive for enteric pathogens (agglutination-confirmed *Salmonella* and/or PCR-confirmed STEC)<sup>16</sup> compared with process water in which these pathogens were not detected. At 1–10 CFU/100 mL, the odds of detecting enteric pathogens were 11-fold the odds when *E. coli* was below such threshold values.

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 FFVs at primary production (including the associated operations of washing/rinsing, sorting, transport, cooling, etc.) 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 such indicators and levels could be regarded as possible performance standards to control the detection of 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 FBOp 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 (RTE or non-RTE FVH, respectively) among others (FAO/WHO, 2021).

In this sector-specific opinion for the frozen 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 '3 × 3 approach' should be followed for each operation in post-harvest processing where water is applied:

<sup>16</sup>Thirty-six out of 37 detected enteric pathogens were *Salmonella*-positive samples, with all 36 confirmed by a Latex agglutination test.



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

As such, nine 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 different 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 operating conditions that were validated, 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 physico-chemical 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. Moreover, none of the scenarios included chlorine-based disinfectant treatments. 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 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 (SGs 2.2 and 4).
- c. For the cooling step after blanching, the temperature of the water reused after cooling needs to be decreased as soon as possible to avoid microbial growth. The temperature decrease needs to be followed up as an operational monitoring activity.

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, (ii) increase of the water replenishment frequency, (iii) correction of the dose of the disinfectant applied or mode of addition (e.g. continuous versus 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 frozen 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 the 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 x 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 case of seasonal production, e.g. spinach and peas, the monthly sampling can be replaced by taking 12 samples over the seasonal production cycle (e.g. 3 months, with a sample every week).

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, 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 need to be done to identify potential systemic issues in the frame of the verification. Table 5 gives an overview of the frequency of trend observations and trend analyses based on the data collected to verify the water management plan.

**TABLE 5** Proposal of frequency for trend observation and trend analysis in verification of the water management plan for the frozen 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 (EFSA BIOHAZ Panel, 2023).

### 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 Appendixes F and G.

## 4 | CONCLUSIONS

This opinion only covers ToR 1.1. as a sub-ToR from ToR1 because industrial data were 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 ToR 2 and ToR 3 were addressed.

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

**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 fFVH?

Industrial data covering 13 scenarios for the frozen 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 10.5 log<sub>10</sub> CFU/100 mL for TBC, from <0 (below LOD) to 8.0 log<sub>10</sub> CFU/100 mL for *E. coli*, and from <0 (below LOD) to 5.8 log<sub>10</sub> CFU/100 mL for *Listeria* spp.
- The hazardous events linked to the handling and/or processing operations using water for the frozen FVH sector include: (i) contaminated water source, (ii) the use of the same water to wash large volumes of product during the operational cycle without a well-managed intervention strategy, (iii) incomplete removal of the water used in the water tank and (iv) improper cooling of process water after blanching.
- Bacterial pathogens were detected in 60 out of 312 water samples analysed, belonging to 7 out of 13 scenarios. *Salmonella* spp. was detected in 7 scenarios and *L. monocytogenes* in 5 scenarios, while STEC was detected in only 1 scenario. Some samples were positive for both *Salmonella* spp. and *L. monocytogenes*.

- When aggregating observations within each sampling visit, *E. coli* levels were higher in process water positive for enteric pathogens compared to process water in which these pathogens were not detected. At 1 to 10 *E. coli* CFU/100 mL, the odds of detecting enteric pathogens was 11-fold the odds of detecting enteric pathogens when *E. coli* was 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.). The variability of the detection of *Salmonella* spp. is mainly due to the scenario whereas of the detection of *L. monocytogenes* mainly due to the sampling time point (nested to sampling visit and scenario) as random effects, respectively.
- 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 13 scenarios for the frozen FVH sector obtained within the frame of the WASTHOP tender showed that:

- Points of attention were identified regarding prerequisite programme practices 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 the 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 water quality, reducing the impact of the organic matter on the efficacy of chlorine.
- When the 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 that of the culturable cells, indicating 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.

- 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 a 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 with the 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 the 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
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
FC	free chlorine
FBOp(s)	food business operator(s)
fFVH	fresh and frozen fruit, vegetables and herbs
FVH	fruit, vegetables and herbs
GMP	good manufacturing practices
GHP	good hygienic practices
ISO	International Organization for Standardization
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
HACCP	Hazard Analysis Critical Control Point
HAV	hepatitis A virus
HOCl	hypochlorous acid
LOD	limit of detection
MI	microbial indicator
NaClO	sodium hypochlorite
OPRP	operational pre-requisite program
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
STEC	Shiga toxin-producing <i>Escherichia coli</i>
SO	Scientific opinion
SOP	standard operating procedures
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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## REQUESTOR

EFSA

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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## APPENDIX A

### Uncertainty analysis

**TABLE A.1** Potential sources of uncertainty related to the industrial data set collected through EFSA's outsourced activities (Gil et al., 2025) for frozen FVH processing and its use.

Source or location of the uncertainty	Nature or cause of the uncertainty
A limited number of EU FBOs (6) who contributed to EFSA's outsourced activities are active in the frozen FVH sector.	The FBOs belonged to only 2 Member States, resulting in an uncertainty that not all practices in processing frozen FVH across Europe were included in the available data set.
A limited number of scenarios (13) in the data set collected through EFSA's outsourced activities for frozen FVH resulted in a limited range of commodities (5 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 frozen FVH across Europe were included in the available data set. For example, most reported food-borne outbreaks mediated by frozen FVH were associated with frozen berries (as vehicles for enteric viruses) (EFSA BIOHAZ Panel, 2023), but none of the scenarios included berries. 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 were 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 also 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 <i>Salmonella</i> spp. and <i>L. monocytogenes</i> colonies were different (PCR versus Latex Agglutination ( <i>Salmonella</i> ) and PCR versus carbohydrate fermentation testing ( <i>L. monocytogenes</i> )). 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 <i>Salmonella</i> , 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 BIOHAZ Panel, 2025a).

Abbreviations: FBO, 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 frozen FVH sector in EFSA's outsourced activities (Gil et al., 2025)

TABLE B.1 Overview of the industrial scenarios for the frozen FVH sector (EFSA outsourced activities – Gil et al., 2025).

Scenario ID code	Water source	Handling/processing operation	Disinfectant type	Food product
49	Recycled water	Water transport	None	Onions
50	Recycled water	Washing	None	Spinach
51	Recycled water	Washing	None	Spinach
52	Recycled water	Washing	None	Spinach
53	Recycled water	Pre-washing	H <sub>2</sub> O <sub>2</sub>	Diced papers
54	Well water	Cooling	H <sub>2</sub> O <sub>2</sub>	Diced papers
55	Municipal tap water	Cooling	PAA	Diced papers
56	Well water	Pre-washing	H <sub>2</sub> O <sub>2</sub>	Diced onion
57	Well water	Washing	H <sub>2</sub> O <sub>2</sub>	Diced onion
58	Municipal tap water	Cooling	PAA	Spinach
59	Municipal water – recycled water	Cooling	PAA	Spinach
60	Municipal tap water	Washing	PAA	Parsley
61	Municipal tap water	Washing	PAA	Chives



## APPENDIX C

## Pathogen-positive findings in process waters of frozen FVH

**TABLE C.1** Pathogen-positive process waters characterised by food product, water source, disinfectant type, sampling visit, sampling time point and mass of frozen FVH processed in the same water (EFSA outsourced activities – Gil et al., 2025).

Scenario ID code	Food product	Handling/ processing operation	Water source	Disinfectant type	Sampling visit number	Sampling time point	Sampling time (min)	Product processed (kg)	Pathogen findings <sup>17</sup>
49	Onions	Water transport	Recycled water	No water treatment	1	1	15	1000	<i>Salmonella</i>
49	Onions	Water transport	Recycled water	No water treatment	1	2	1155	143,000	<i>Salmonella</i>
49	Onions	Water transport	Recycled water	No water treatment	1	4	2595	308,000	<i>Salmonella</i>
49	Onions	Water transport	Recycled water	No water treatment	2	2	800	116,234	<i>Salmonella</i> , <i>L. monocytogenes</i>
49	Onions	Water transport	Recycled water	No water treatment	2	4	3215	286,787	<i>Salmonella</i>
49	Onions	Water transport	Recycled water	No water treatment	2	5	3380	308,730	<i>Salmonella</i>
50	Spinach	Washing	Recycled water	No water treatment	1	5	5950	930,000	<i>Salmonella</i>
50	Spinach	Washing	Recycled water	No water treatment	2	1	0	0	<i>Salmonella</i>
50	Spinach	Washing	Recycled water	No water treatment	2	2	905	99,087	<i>L. monocytogenes</i> , <i>Salmonella</i>
50	Spinach	Washing	Recycled water	No water treatment	2	3	1390	160,355	<i>Salmonella</i>
50	Spinach	Washing	Recycled water	No water treatment	2	6	5200	609,149	<i>L. monocytogenes</i> , <i>Salmonella</i>
51	Spinach	Washing	Recycled water	No water treatment	1	3	1570	194,000	<i>L. monocytogenes</i>
51	Spinach	Washing	Recycled water	No water treatment	1	4	2070	269,624	<i>L. monocytogenes</i>
51	Spinach	Washing	Recycled water	No water treatment	1	5	2985	411,385	<i>L. monocytogenes</i>
51	Spinach	Washing	Recycled water	No water treatment	1	6	4470	628,203	<i>L. monocytogenes</i> , Norovirus
51	Spinach	Washing	Recycled water	No water treatment	2	1	185	27,057	<i>L. monocytogenes</i>
51	Spinach	Washing	Recycled water	No water treatment	2	2	700	99,209	<i>L. monocytogenes</i>
51	Spinach	Washing	Recycled water	No water treatment	2	3	1630	232,240	<i>L. monocytogenes</i>
51	Spinach	Washing	Recycled water	No water treatment	2	4	2225	329,650	<i>L. monocytogenes</i> , <i>Salmonella</i>
51	Spinach	Washing	Recycled water	No water treatment	2	5	3070	455,303	<i>L. monocytogenes</i>
51	Spinach	Washing	Recycled water	No water treatment	2	6	4510	660,621	<i>L. monocytogenes</i>
52	Spinach	Washing	Recycled water	No water treatment	1	2	960	90,419	<i>Salmonella</i> , STEC
52	Spinach	Washing	Recycled water	No water treatment	1	3	1410	149,512	<i>Salmonella</i>
52	Spinach	Washing	Recycled water	No water treatment	1	4	2390	274,245	<i>Salmonella</i>
52	Spinach	Washing	Recycled water	No water treatment	1	6	3960	431,713	<i>Salmonella</i>

<sup>17</sup>Confirmation of *Salmonella* was performed by agglutination testing. STEC was confirmed by PCR. Confirmation of *L. monocytogenes* was performed by carbohydrate fermentation testing. The presence of norovirus GI and GII was detected by RT-qPCR.

TABLE C.1 (Continued)

Scenario ID code	Food product	Handling/ processing operation	Water source	Disinfectant type	Sampling visit number	Sampling time point	Sampling time (min)	Product processed (kg)	Pathogen findings <sup>17</sup>
52	Spinach	Washing	Recycled water	No water treatment	2	1	110	10,850	<i>L. monocytogenes</i>
52	Spinach	Washing	Recycled water	No water treatment	2	2	365	43,675	<i>L. monocytogenes, Salmonella</i>
52	Spinach	Washing	Recycled water	No water treatment	2	3	1575	188,801	<i>Salmonella</i>
52	Spinach	Washing	Recycled water	No water treatment	2	5	1980	241,347	<i>L. monocytogenes</i>
52	Spinach	Washing	Recycled water	No water treatment	2	6	2165	275,944	<i>L. monocytogenes, Norovirus</i>
53	Diced peppers	Pre-washing	Recycled water	H2O2	1	6	190	18,455	Norovirus <sup>18</sup>
53	Diced peppers	Pre-washing	Recycled water	H2O2	2	6	75	7285	Norovirus <sup>12</sup>
55	Diced onions	Cooling	Municipal tap water	PAA	1	1	30	1000	<i>Salmonella</i>
55	Diced onions	Cooling	Municipal tap water	PAA	1	5	2790	343,000	<i>Salmonella</i>
55	Diced onions	Cooling	Municipal tap water	PAA	2	2	790	116,234	<i>Salmonella</i>
55	Diced onions	Cooling	Municipal tap water	PAA	2	3	1820	244,493	<i>Salmonella</i>
55	Diced onions	Cooling	Municipal tap water	PAA	2	4	3200	286,787	<i>Salmonella, L. monocytogenes</i>
55	Diced onions	Cooling	Municipal tap water	PAA	2	5	3370	308,730	<i>Salmonella</i>
58	Spinach	Cooling	Municipal tap water	PAA	1	6	6940	1,055,000	Norovirus
58	Spinach	Cooling	Municipal tap water	PAA	2	5	3840	470,146	<i>Salmonella</i>
58	Spinach	Cooling	Municipal tap water	PAA	2	6	5255	609,149	Norovirus
59	Spinach	Cooling	Municipal + recycled water	PAA	1	1	0	0	<i>Salmonella</i>
59	Spinach	Cooling	Municipal + recycled water	PAA	1	4	2370	274,245	<i>Salmonella</i>
59	Spinach	Cooling	Municipal + recycled water	PAA	1	6	3930	431,713	Norovirus

<sup>18</sup>Intact capsid was assessed by RT-qPCR after PMAxx pretreatment and RNA extraction in Norovirus GI-positive samples.

TABLE C.1 (Continued)

Scenario ID code	Food product	Handling/ processing operation	Water source	Disinfectant type	Sampling visit number	Sampling time point	Sampling time (min)	Product processed (kg)	Pathogen findings <sup>17</sup>
59	Spinach	Cooling	Municipal + recycled water	PAA	2	1	95	10,850	<i>Salmonella</i>
59	Spinach	Cooling	Municipal + recycled water	PAA	2	6	2190	275,944	Norovirus
61	Chives	Washing	Municipal tap water	PAA	2	6	305	7582	Norovirus

## APPENDIX D

**Relationship between the occurrence of potential microbial indicator groups above a given threshold and the detection of *L. monocytogenes* or enteric pathogens (*Salmonella* spp. or STEC)****TABLE D.1** Odds ratio (OR) for the detection of *L. monocytogenes* in process water from the frozen 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.

Bacterial indicator group and its threshold	Data concerning frozen 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)
TBC threshold (CFU/100 mL)						
10 <sup>4</sup>	– <sup>a</sup>			– <sup>a</sup>		
10 <sup>5</sup>	– <sup>a</sup>			– <sup>a</sup>		
10 <sup>6</sup>	– <sup>a</sup>			<b>12.2</b>	2.6–58.3	0.002
TC threshold (CFU/100 mL)						
10 <sup>2</sup>	– <sup>a</sup>			<b>8.6</b>	1.1–68.7	0.042
10 <sup>3</sup>	– <sup>a</sup>			<b>12.7</b>	1.6–101.4	0.016
10 <sup>4</sup>	– <sup>a</sup>			<b>5.0</b>	1.3–19.4	0.019
<i>E. coli</i> threshold (CFU/100 mL)						
1 <sup>b</sup>	– <sup>a</sup>			<b>7.4</b>	1.6–35	0.012
10	– <sup>a</sup>			<b>11.7</b>	2.4–55.7	0.002
100	– <sup>a</sup>			<b>26.0</b>	5.3–127.4	<0.001
<i>Listeria</i> spp. threshold (CFU/100 mL)						
1 <sup>b</sup>	– <sup>a</sup>			<b>5.8</b>	1.2–27.6	0.026
10	– <sup>a</sup>			<b>11.2</b>	2.3–53.2	0.003
100	1.2	0.2–7.6	0.830	<b>5.2</b>	1.6–17.1	0.007

Note: Bold: indicates statistically significant differences.

Abbreviation: CI, confidence interval.

<sup>a</sup>OR could not be calculated because *L. monocytogenes* was not detected in any sample with microbial indicator group levels below the threshold.

<sup>b</sup>Limit of detection.

**TABLE D.2** Odds ratio (OR) for the detection of **enteric pathogens** (agglutination-confirmed *Salmonella* and/or PCR-confirmed STEC)<sup>19</sup> in process water from the frozen FVH sector for different thresholds of TBC, TC, *E. coli* and *Listeria* spp. counts as 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.

	Data concerning frozen 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)
TBC threshold (CFU/100 mL)						
10 <sup>4</sup>	– <sup>a</sup>			<b>12.7</b>	<b>1.6–98.3</b>	<b>0.0151</b>
10 <sup>5</sup>	2.8	0.4–18	0.285	<b>8.5</b>	<b>2.3–30.4</b>	<b>0.001</b>
10 <sup>6</sup>	2.8	0.4–18	0.285	<b>9.8</b>	<b>3.3–29.2</b>	<b>&lt;0.0001</b>
TC threshold (CFU/100 mL)						
10 <sup>2</sup>	6.1	0.6–62.2	0.126	<b>18.9</b>	2.4–146.2	<b>0.005</b>
10 <sup>3</sup>	6.1	0.6–62.2	0.126	<b>13.3</b>	3–60.1	<b>0.0008</b>
10 <sup>4</sup>	6.1	0.6–62.2	0.126	<b>22.1</b>	4.9–100.2	<b>&lt;0.001</b>
<i>E. coli</i> threshold (CFU/100 mL)						
1 <sup>b</sup>	<b>11.0</b>	1.1–109.7	0.041	<b>11.0</b>	3.1–39.9	<b>&lt;0.001</b>
10	<b>11.0</b>	1.1–109.7	0.041	<b>4.1</b>	1.6–10.8	<b>0.004</b>
100	2.0	0.4–9.8	0.394	2.3	0.9–6.2	<b>0.086</b>

<sup>19</sup>Thirty-six out of 37 detected enteric pathogens were *Salmonella*-positive samples, with all 36 confirmed by a Latex agglutination test.

TABLE D.2 (Continued)

	Data concerning frozen FVH sector			Complete data set for all sectors (fresh-whole, fresh-cut and frozen FVH)		
	OR	95% CI	Significance level ( <i>p</i> )	OR	95% CI	Significance level ( <i>p</i> )
<i>Listeria</i> spp. threshold (CFU/100 mL)						
1 <sup>b</sup>	– <sup>a</sup>			<b>30.5</b>	3.9–235.7	<b>0.001</b>
10	– <sup>a</sup>			<b>12.2</b>	3.8–39.4	< <b>0.001</b>
100	0.4	0.1–1.9	0.230	2.2	0.8–6	<b>0.118</b>

Note: Bold: indicates statistically significant differences.

Abbreviation: CI, confidence interval.

<sup>a</sup>OR could not be calculated because *L. monocytogenes* was not detected in any sample with microbial indicator group levels below the threshold.

<sup>b</sup>Limit of detection.

## APPENDIX E

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

**TABLE E.1** Input parameter values for model simulations of all the scenario groups (SGs) of the frozen FVH sector.

Parameter	Value	Units	Description	Source for the value (reference)
$\alpha$	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)
$\beta$	$7.81 \times 10^{-4}$	1/(mg-COD/L·min)	Rate constant of reaction FC and COD	Estimated value (Gil et al., 2025)
$\gamma$	8.16	mg-COD/mg-FC	Yield coefficient COD of reaction FC and COD	Estimated value (Gil et al., 2025)
$\lambda$	$1.70 \times 10^{-3}$	1/min	Natural decay of FC	Retrieved from Abnavi et al. (2021)
$n$	1	–	Exponent in Hill equation	Assumed (Gil et al., 2025)
$K_m$	$3.22 \times 10^3$	mg-COD/L	Protective COD effect	Estimated value from data collected through EFSA's outsourced activities (Gil et al., 2025)
$K_x$	$2.92 \times 10^6$	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)
$K_{COD}$	$2.61 \times 10^2$	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)

**TABLE E.2** Input data for variables in model simulations of all the scenario groups (SGs) of the frozen FVH sector.

Variables	Value	Units	Description
$V$	2500	L	Tank water volume
$\tau$	2	min	Contact time
$P$	10,192	Kg	Total product added
$COD_i$	0	mg-COD/L	Initial COD in water (mg/L)
$X_{wi}$	0	CFU/100 mL	Initial bacterial contamination level (CFU/mL)









**ANNEX A**

**Excel file including the full data set collected during the sampling visits to all FBOs 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.