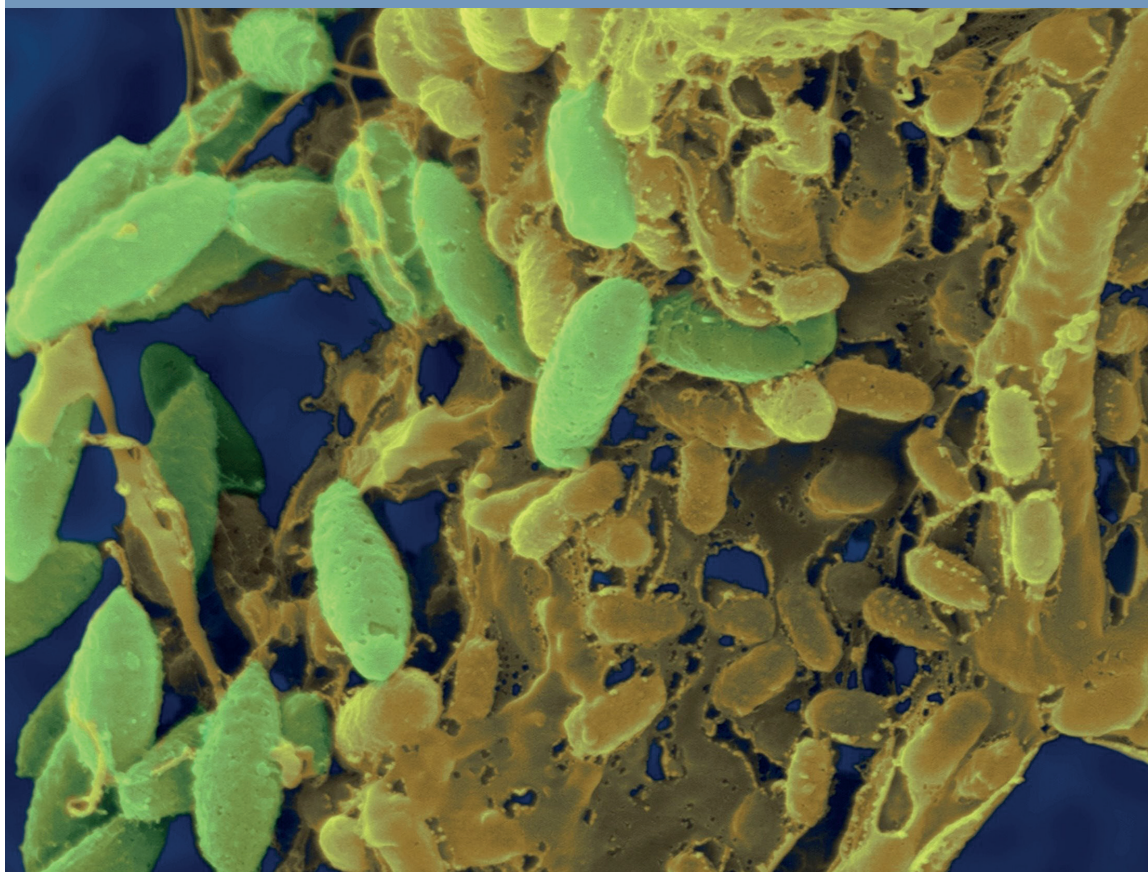


Prevention and Control of Microbiological Contamination in Crop Protection Products



Picture Front Cover

Colored scanning electron micrograph of a biofilm on a stainless steel surface. Biofilms are an accumulation of microorganisms encapsulated in a matrix and attached to moist surfaces (high humidity often suffices)¹.

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The “[Prevention and Control of Microbiological Contamination in Crop Protection Products](#)” booklet makes recommendations about best practices to prevent and control Product Integrity incidents caused by microbial contamination.

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Foreword

January 2018

Dear Reader,

The world needs farmers, and farmers need Plant Science. Plant Science provides modern agriculture with the tools and technologies that enable farmers to grow healthy produce in a safe manner and at affordable prices. One of the critical success factors to achieve this is to ensure Crop Protection Products (CPPs) have the highest possible quality .

The remit of the CropLife International Operations Committee is to ensure our member companies, with our external manufacturers, have effective manufacturing procedures for consistent production of quality CPPs.

In view of the potentially far reaching consequences of contamination of products during the manufacturing process, either by microorganisms or by extraneous chemicals, this aspect continues to require a high level of awareness and attention.

This revision to the “Prevention and Control of Microbial Growth in Water-based Crop Protection Formulations”, includes the very latest guidelines and best practices to further optimize prevention and control of microbiological contamination in CPPs.

On behalf of the CropLife International Operations Committee, I hope you will find this new and completely revised booklet “Prevention and Control of Microbiological Contamination in Crop Protection Products” an up-to-date tool to help prevent and/or control contamination incidents caused by microorganisms.

Susan Lewis

Chair, CropLife International Operations Committee

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1. Introduction

The presence of microorganisms in water-based crop protection products may have a negative effect on the product quality. Such a situation is commonly referred to as microbial contamination.

Harmful microorganisms can cause a number of changes of the physical properties of the contaminated formulation. Changes may affect pH, viscosity, dispersion stability, phase separation and sedimentation, which negatively impact the storage stability and application of the product. A further, highly undesirable effect can be the decomposition of the active ingredient(s) and co-formulants.



Fig. 1: Phase separation (left) followed by precipitation



Fig. 2: Bulging containers due to gas production from microbial contamination

Microbial growth may cause bulging containers due to generation of gas, which often has a foul odor.

It is highly important that all these quality issues are addressed to ensure the products meet all specified requirements and give full customer satisfaction. As it is unrealistic to expect aseptic conditions in the manufacturing facilities of crop protection products, the objective is to prevent microbial growth during all steps of the manufacturing process through strict hygiene management. Additionally, adding a registered biocide to the finished product may be necessary.

Equally important in assuring quality products is the prevention of the contamination of crop protection products caused by extraneous chemicals (often from products previously present in the equipment). Detailed guidelines and best practices dealing with these aspects can be found in the CropLife International booklet “Contamination Prevention in the Manufacture of Crop Protection Products” (2014)².

In the current booklet the convention adopted throughout follows the International Standards Organization definitions:

- **Requirements:** *shall, shall not, must, have to.*
- **Recommendations:** *should, should not.*
- **Permission:** *may, need not.*
- **Possibility and capability:** *can, cannot.*

² Electronic copy: http://croplife.org/wp-content/uploads/pdf_files/CPMCP-PP-LR_e-version_14_Aug2014.pdf

2. Purpose and Scope

The purpose of this booklet is to provide guidance on the prevention and control of microbial contamination to all manufacturers of crop protection products. It is applicable to: synthesis of intermediates and the active ingredients, formulations and (re)-packaging, procurement and warehousing of raw materials and packaging materials. The use of biocides for protection of the finished product against development of microbial growth during storage will be discussed. Microbiological test methods, cleaning procedures of the plant equipment and optimized choice of disinfection products will be described.

The following crop protection chemical products fall under the scope of this booklet, either as formulated products, active ingredients or process intermediates:

Formulation types

- Suspension concentrate (SC).
- Suspo-emulsion (SE).
- Soluble concentrate (SL).
- Flowable concentrates for seed treatment (FS).
- Microencapsulated products (CS).
- Gel, for direct application (GD).
- Baits.

Excluded from the scope of this document are:

- Microbial and biological pesticides (see OECD guideline 43³).
- Prevention or control of microbial contamination of application equipment at farm or contract applicators level.
- Cleaning of equipment and storage facilities in biotechnology and seed operations.
- Cleaning operations at retail bulk storage facilities and cleaning of refillable containers.

These guidelines are globally applicable to all CropLife International member companies, and to their current and potential future external manufacturers. Suggestions will be made regarding which minimum Microbial Contamination Prevention standards should form part of contract manufacturing agreements with external manufacturers to ensure the required product quality.

³ OECD Guideline OECD ENV_JM_MONO (2011) 43

3. Requirements for Prevention and Control of Microbial Contamination

The intent of this chapter is to provide the requirements that will mitigate the risks of microbial contamination associated with manufacturing water-based products.

All CropLife International member companies and their external manufacturers shall commit to the Policy and Requirements in this chapter.

This policy does not replace the policy published in Chapter 4 of the “Contamination Prevention in the Manufacture of Crop Protection Products” booklet which is aimed at the prevention of chemical cross-contamination. Where appropriate both policies have to be implemented.

3.1 Microbial Contamination Prevention and Control Policy

- Member companies shall ensure that their products will be either free of microbial contamination or have a controlled level of microbial growth that will not compromise quality, safety, and/or efficacy.
- The individual member companies shall set the limits for their products.
- Regulatory requirements, local legislation and other official regulations must be implemented and followed at all times.
- A company which uses an external manufacturer (EM) shall provide this EM with available information to enable appropriate risk assessment and to develop effective microbial contamination prevention and control procedures.

3.2 General Requirements

- Documented risk assessments for microbial contamination prevention and control must be in place.
- Raw materials and their handling must be assessed.
- Acceptable levels of Colony Forming Units (CFUs) for finished products must be defined.
- Refillable containers (IBCs, ISOs, Big Bags, Rail Trucks, etc.) must be cleaned in the same way as equipment that comes into contact with the products.
- Recycling and reprocessing must be managed to minimize any risk of microbial contamination.
- To prevent microbial growth the use of non-registered biocides or registered biocides exceeding registered limits is forbidden.
- All materials must be clearly and properly labelled, this includes but is not limited to: raw materials, intermediates, bulk formulations, finished products,

- product to be reprocessed, recycle, and waste.
- Effective cleaning procedures and analytical methods must be available to determine the CFUs in rinsates prior to the manufacturing campaign and/or in the finished product.
 - A cleaning operation followed by drying must take place as soon as possible after production has stopped, irrespective whether a succeeding product is scheduled for that particular equipment. This stops possible build-up of micro-organisms in the equipment.

3.3 Management Responsibilities

The management of all CropLife International member companies and their EMs shall ensure the following responsibilities and requirements are covered and implemented:

- Protect the confidentiality of exchanged information.
- Provide sufficient resources for all aspects of Prevention and Control of Microbial Contamination.
- Apply requirements and best practices as demonstrated in this booklet.
- Continuous training and awareness.
- Effective hygiene and good housekeeping.

Exceptions to any of the general requirements must have documented approval from senior management.

3.4 External Manufacturing of water-based Products

3.4.1 Information Exchange

Before starting the agreed production, the External Manufacturer must supply, in a timely manner, the following information to their client.

- Configuration of the production unit in which the product will be synthesized, formulated and/or filled. Ensure that the configuration shall be cleaned and sanitized to the required levels.
- Parallel operations with emphasis on the degree of segregation, common equipment and personnel.
- The precise location of the production facility (e.g., GPS coordinates).

The client must supply to the external manufacturer methods to determine the level of the microbial contamination and effectiveness of the required cleaning and sanitation procedures. The External Manufacturer is required to verify that the cleaning methods are effective in their plant, equipment and configuration.

3.4.2 Minimum Requirements for External Manufacturers

In addition to the guidelines detailed above, it is expected that the items below are incorporated in the agreement / contract between the client and the EM.

The client is responsible:

- To define the acceptable microbial contamination level in the finished product.
- To undertake detailed site audits and other preventive measures (including risk assessment, cleaning procedures), and support the EM where appropriate.
- To inform the EM of any special risks associated with the product being brought onto its site.
- To review and if necessary update existing contracts and/or agreements with EMs to include the best practices as outlined in this document.

The EM is responsible:

- To cooperate in audits for microbial contamination prevention.
- To track materials and retain all relevant records as defined by the client to enable traceability.
- To appoint a person responsible for implementation of the Prevention and Control of Microbial Contamination Guidelines at the EM's site.
- To ensure adequate analytical capability is available.
- To ensure there are written clean-out procedures and checklists to be followed.
- To ensure periodic microbial contamination prevention training of existing personnel, maintenance staff, newly recruited personnel, as well as, temporary employees before they are allowed to participate in the manufacturing process, and that records of the training are retained.
- To ensure permanent labeling of all equipment (including portable equipment) and containers for: raw materials, in-process materials, finished products, and waste.
- To obtain approval from the client prior to any change that impacts the risk of microbial contamination.
- To ensure that samples are not recycled, i.e., samples shall not be returned in the process.
- To ensure that any reprocessing (incl. rework, blending, recycling) is approved by the client.
- To maintain good housekeeping practices.
- To ensure the retention time and storage conditions of retained samples specified by the client are followed.

3.5 Procurement / Purchasing of active ingredients, intermediates and raw materials

Products purchased from suppliers of active ingredients (AIs), intermediates and/or raw materials must meet all quality criteria, including those related to prevention and/or control of microbial contamination.

Protection of a supplier's intellectual property remains important. Therefore it is recommended to implement a secrecy agreement. The business partners should agree in the contract to implement all requirements listed in the booklet.

As a minimum the following aspects related to microbial contamination should be covered in the supply contract:

- Definition of microbial contamination and contamination prevention (as described in this booklet; see Glossary)
- Agreement reached that:
 - o Disclosure of microbiological analysis data (including analytical methods) and an agreed list of parameters on the certificate of analysis.
 - o Notification of process changes as required.

These aspects are additional to, and complement, the requirements laid out in the "Contamination Prevention in the Manufacture of Crop Protection Products, Guidelines and Best Practices" (see footnote page 10).

4. Microorganisms responsible for potential contamination and their biological requirements

The microorganisms that can potentially cause contamination of water-based products belong to two distinct biological kingdoms: **Bacteria and Fungi**.

To help understand the logic behind the measures and best practices required for effective prevention and control of microbial contamination, information will be given on the biology of microorganisms, their sources, and the factors affecting microbial growth.

4.1 Bacteria

Bacteria are small, typically single cell organisms. They are not visible with the naked eye and require a microscope.

Typically bacteria have a length / diameter of a few microns. Depending on the species, the shape ranges from spheres, rods, to spirals, with or without a flagellum or pili for locomotion. Bacteria often attach themselves to surfaces to form complex structures known as biofilms. Biofilms will be discussed in more detail later because they can present a major challenge in the cleaning of production units.

Examples of some common bacterial species encountered in our industry include *Bacillus* and *Pseudomonas*.

Bacteria can be found nearly everywhere. In manufacturing of water-based crop protection products, they can be present in raw materials, process water, and packaging components, as well as on the in- and outside of process equipment, in the air, on the walls, floors and ceilings of production units, and on pallets, etc.

4.1.1 Bacterial Growth

Bacteria reproduce by binary fission, i.e., the cell reproduces itself and breaks into two genetically identical cells.

Four phases can be recognized in the growth curve (life cycle) of bacteria (see fig. 3):

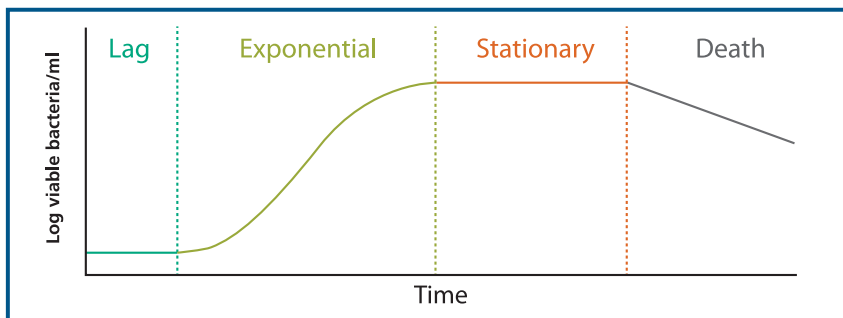


Fig. 3: Schematic presentation of a typical bacterial growth curve

- **The lag phase:**

During this phase, freshly introduced bacteria will only resume a full growth rate and cell divisions after adaptation to the newly available, often different nutrients and/or altered environmental conditions.

- **The exponential or logarithmic phase:**

Once the bacteria show a constant growth rate, they have entered the exponential or logarithmic phase. During this phase, all cells are viable and duplicate themselves (i.e., divide) at a very high rate. The reproduction rate is species specific and depends on the actual environment. Under ideal conditions the number of bacterial cells doubles in as little as 20 to 30 minutes. This explosive multiplication of cells accounts for the fact that the problems associated with microbial contaminations can occur very quickly.

- **The stationary phase:**

The viable cell count remains constant in this phase. Although cell division will continue during this phase, due to an increased number of dead cells the population growth stopped. This phase can last for hours, even days.

- **The death phase:**

When the nutrients or environmental factors become limiting factors, the population enters the death phase. This is an exponential process with a high death rate.

However, a small number of survivors often persist for months or even years and have the potential to eventually establish a new population, especially when the contaminating species form endospores.

4.1.2 Resistance

Modification of the genetic make-up of bacteria is caused by mutations (errors in duplication of the genetic material, DNA). This can help the bacteria to respond to environmental changes that slow down the growth of the population, e.g., depletion of nutrients, the presence of biocides.

4.1.3 Endospores

Under unfavorable environmental conditions certain genera of bacteria, e.g., *Bacillus*, *Clostridium*, will form highly resistant, dormant endospores which can remain dormant for very long periods of time. During this phase the metabolic processes are on hold.

The endospores are surrounded by an impermeable, rigid coat and can, as a consequence, survive under extreme physical and chemical stress: UV light, heat, freezing, pressure, desiccation, disinfectants, detergents and organic solvents.

The danger exists that endospores can become airborne and this could trigger a wide-spread distribution in the entire manufacturing environment.

The potential contamination by endospores requires serious consideration when developing strategies for plant hygiene and plant design.

4.2 Fungi

The fungi encountered in the manufacturing environment can be classified in two groups: **Yeasts and Molds**.

Also in fungi, mutations can occur allowing adaptations to environmental changes. The growth rate can be high, depending on the right nutritional and environmental conditions.

4.2.1 Yeasts

Yeasts are small, micron sized single-cellular organisms and typically 3 – 4 microns in diameter although some species reach a diameter of 40 microns.

Yeasts reproduce by a process called budding. The newly formed cells are released into the environment as single cell, but in some species, they may remain attached to the “parent” cell.

4.2.2 Molds

Molds are fungi which grow by forming multicellular, tubular hyphae which form a network referred to as mycelium. Sometimes mycelia form large mats (colonies) on the surface of water-based formulations and these are visible with the naked eye.

Depending on environmental conditions, especially when it is warm and humid, molds produce spores which can become airborne. These spores become freely distributed in the environment with the possible result that they contaminate products, raw materials, manufacturing equipment etc.

4.3 Biofilm

A biofilm is a group of microorganisms in which bacterial – or bacterial and fungal (and sometimes algal) cells adhere to each other and stick to a surface, or in the case of a plastic substrate even grow into the polymer. Typically, rough surfaces, cracks and deposits are the first attachment points for microbes.

The microbial cells are embedded within a self-produced matrix containing extracellular DNA, enzymes, other proteins, polysaccharides, metabolites and water. Often the matrix will also contain the chemical product being made in the infested equipment which may serve as nutrient source.

Microorganisms inside a biofilm are much harder to control than microbes that float or swim in the outside liquid environment. There are two reasons for this phenomenon:

- The metabolism of cells inside the biofilm differs from that of single cells of the same species living outside the biofilm matrix in the liquid medium.
- The matrix provides protection against outside factors.

For example, levels of sodium hypochlorite (e.g., 1 - 3 ppm as Cl_2) that are sufficient to kill most microbes in water will have little, if any, effect on those same microbial species in a biofilm. Other chemicals like H_2O_2 , peracetic acid or biocides are usually not able to remove an existing biofilm completely; only the outer areas might be removed. This means it is nearly impossible to remove a biofilm when already formed.

An additional complication is that once a biofilm becomes established, it will release free floating microbes. These will spread further downstream in the equipment and form a new biofilm (Fig. 4). This also applies to “flakes” of biofilm broken off during mechanical cleaning.

Undesirable side-effects of biofilms are bio-corrosion and/or bio-fouling (formation of blockages/plugs and leakages).

During the initial stages of their development biofilms cannot be seen with the naked eye, however detection of the initially transparent slime layer on the surface is possible by simple contact with the fingers. Later growth stadia are detectable visually. Usually swab testing is the most reliable method to confirm the presence of biofilms, especially at an early growth stage.

This section shows that contamination with biofilms is very serious because disinfection is difficult and not completely effective. In Chapter 6 (6.2, 6.7.1), a possible treatment strategy will be discussed.

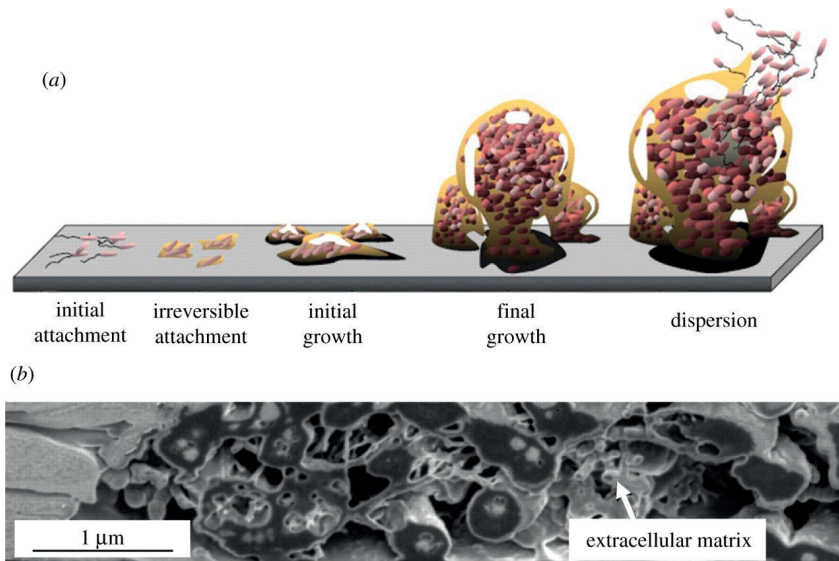


Fig. 4: Biofilm development⁴, (a) five-stage colonization process and (b) cross-section of a mature colony - showing the extracellular matrix and water channels (Scanning Electron Microscopy).

4.4 Conditions for Microbial Growth

Optimal or near optimal conditions for microbial growth often are present in the manufacturing facilities for water-based crop protection products. Knowledge of the growth requirements of microorganisms is essential to develop an effective defense strategy.

4.4.1 Water

For microorganisms water in the liquid phase is not always necessary to support growth. With some species found in the manufacturing environment, microbial growth can occur at high relative humidity (RH) (i.e., water in the vapor phase). For example, this can occur with solid products inside production units.

4.4.2 Atmospheric Conditions (Aerobe /Anaerobe)

There are three types of microorganisms:

- Aerobic organisms grow in an oxygenated atmosphere.
- Anaerobes do not require oxygen to sustain growth.
- Facultative anaerobes, they will use oxygen when available, but they are able to switch to fermentation or anaerobic respiration if the oxygen concentration will decrease.

⁴ Credit: Bixler G.D., Bhushan, B. (2012). Biofouling: lessons from nature. *Phil. Trans.R. Soc. A* **370**, 2381-2417. DOI:1098/rsta.2011.0502.

4.4.3 Nutrients

The bacteria and fungi responsible for microbial contaminations all depend on extracellular material (nutrients) for their metabolism. Carbon and nitrogen need to be available in relatively large quantities to sustain microbial growth, while phosphorous and sulfur are required in smaller amounts. The various trace elements are normally present in sufficient quantities as contaminants of crop protection products.

4.4.4 Temperature

Formulation plants are typically operated at a temperature range between 10 °C and 40 °C. These temperatures provide favorable conditions for the growth and reproduction of the microorganisms commonly found in these facilities.

The lower limit for the growth of microbes is generally slightly below 0 °C. However, many micro-organisms can survive in a frozen state for prolonged periods, albeit that all metabolic processes have been shut down.

The upper limit of the growth is determined by the thermo-stability of the proteins and nucleic acids in the cytoplasm. These molecules are destroyed mostly between 60 °C and 90 °C. Exposure to temperatures above the growth range causes rapid cell death. This forms the basis for heat sterilization. A cause for concern form the spore-forming microbes for the spores, especially endospores, can survive much higher temperatures than vegetative cells.

4.4.5 pH

Yeasts and molds will display optimal growth in an environment with a pH ranging from slightly acid to neutral.

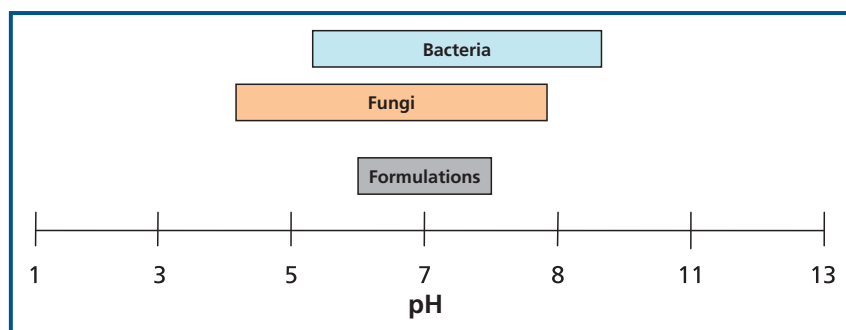


Fig. 5 The typical pH ranges for bacterial and fungal growth and formulations are shown above.

In general, fungi grow better at lower pH values than bacteria. Most bacteria are relatively insensitive to the external concentration of H^+ and OH^- ions and many species can grow well at any pH value between 6.0 and 9.0⁵. In conclusion, the pH values of most water-based crop protection products fall within a range which can sustain the growth of both bacteria and fungi common in production sites (Fig. 5).

⁵ *The explanation is that cell walls of living cells are only slightly permeable to H^+ and OH^- ions leaving the "internal" pH in the desired neutral range.*

5. Sources and Prevention of Microbial Contamination

The design of a manufacturing facility, the raw materials, recycling procedures, the atmospheric conditions in the plant (air quality, temperature and relative humidity), the presence of free-standing water, dust, the ease with which the units can be sterilized, the housekeeping, the approach of the staff to personal hygiene are all factors determining the environment in which microorganisms can grow. There may be additional factors, this is not meant to be an exhaustive list.

5.1 Quality Criteria for Water

Water is the single most important, microbiologically critical raw material in the manufacture of water-based crop protection products. All sources of water have microorganisms at different levels depending on its origin:

- Potable / Tap water.
- Well water.
- Rain water.
- Surface water (rivers, lakes, etc.).
- Water from central water preparation stations.
- Cooling water /condensed steam.

5.1.1 Preparation of Water for use in manufacturing (Process Water)

To prevent microbial growth in water-based crop protection products, it is essential that the process water, often the raw material with the highest content in the finished product, should ideally be free of bacteria and fungi. However, unless sterile water is used in the manufacturing process, process water will not be entirely free of microbes.

How many microorganisms a manufacturer can tolerate in the process water is a decision specific for each individual company.

As an example, the cosmetic industry uses a threshold of < 1 CFU/100 ml, as stated in the Cosmetic GMP guidelines.

There are no fixed rules regarding the design of the water treatment facilities. Depending on the local situation on the manufacturing site (e.g., water source), the following steps, applied either combined or individually, will help to achieve high quality process water (Fig 6).

- The incoming water (i.e., from the source) should be sampled to check the level of microbial contamination.
- The incoming water is filtrated through a series of three filters with a pore diameter of 25 µm, 5 µm and 1 µm, respectively.

(The text of 5.1.1. continues on page 26)

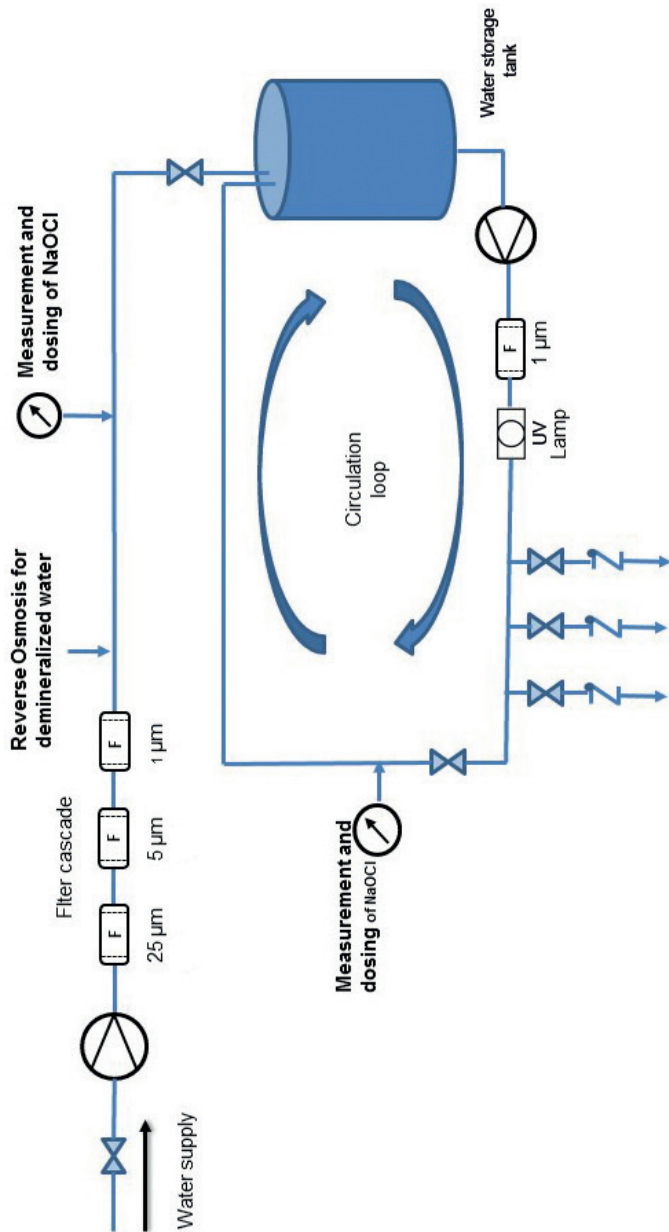


Fig. 6: Schematic flow chart of the various recommended steps in the preparation of water for use in manufacturing (Process Water), including use of a closed loop holding tank.

Pre - treatment of incoming water	Storage	Transfer loop to vessels
<ul style="list-style-type: none"> Water source: tap water, well water, or surface water 3 step particle filtration: 25 μm, 5 μm and 1 μm before water is charged to storage vessel. Possible Reverse Osmosis to obtain demineralized water. 	<ul style="list-style-type: none"> NaOCl dosing system (~ 2 to 3 ppm free Chlorine in treated water) and storage tank; circulation/transfer pump(s). Storage tank should be made of Chlorine stable steel; no plastic. Circulation pump has to run continuously. Water from storage tank to be used for cleaning and rinsing as well. 	<ul style="list-style-type: none"> Water transfer from storage tank via 1 μm filter (safeguard for UV lamp) and UV lamp Circulation loop to be installed to avoid dead ends in the plant area. Loop etc. should be made from Chlorine stable steel, no plastic. Best practice: second measurement and adjustment of NaOCl end of the loop. Non-return valves (back- flush valves) should be installed in the water supply from the loop to the formulation vessels.

- The filtered water is then treated with NaOCl. The maximum free Cl_2 content shall not exceed 5 ppm (5 mg/l), the WHO Guideline value for free chlorine in drinking-water ⁶.
- The typical range used in manufacturing is 1 - 3 ppm of free Cl_2 .
- The optimal pH should be neutral.
- The process water is preferentially treated in-line, however, treated water could be stored in a holding tank after determining the level of microbes. The holding tank could be part of a closed loop with piping branching off to the formulation vessels that have to be fed with the process water.
- Stored water will be filtered once more through a 1 micron filter before UV irradiation.
- Prior to entering the formulation vessels the water undergoes UV treatment, and the water should be checked for microbes both before and after the UV treatment just prior to staging.
- After feeding the vessels, the free chlorine content should be measured and re-adjusted if necessary.

5.1.2 Water used for Equipment Cleaning

Water is the common solvent for all cleaning agents and also for the intermediate and final rinses of the equipment. The chemical and microbiological quality of the “cleaning/rinsing” water is as important as that of process water and should be sourced and prepared in a similar way.

Generally, a pH range from 5 to 8.5 is suitable for most detergents and disinfectants. Highly alkaline or highly acidic water may require additional buffering agents.

Hard water leads to precipitation of insoluble salts which form scales and reduces the effectiveness of detergents. This should be avoided because the scales harbor microorganisms which may form biofilms.

Reduction in the formation of scales can be achieved by addition of chelating and sequestering agents, which bind calcium and magnesium. If detergents or chelating agents are used for cleaning, then a final rinse with process water is necessary.

5.2 Raw materials

Microorganisms can be introduced in the manufacturing environment via contaminated raw materials. The microbiological status of a raw material depends on its nature, origin and manufacturing process, as well as transport, packaging

⁶ WHO (2011) *Guidelines for drinking water quality 4th edition. Chapter 12: Chemical fact sheets: 334.*

and storage conditions. Contact with water and air is especially important. Ideally requirements for a necessary microbial status of raw materials should be laid down in specification agreements.

5.2.1 Critical Raw Materials

Raw materials known to enhance microbial growth include:

- Sugars and cellulose.
- Starch and flour.
- Clay, e.g., kaolin or bentonite.
- Natural pigments.
- Water-based solutions of:
 - o Glycols and polyglycols,
 - o Fatty acid alcohols,
 - o Antifoaming agents,
 - o Synthetic sugars,
 - o Surfactants,
 - o Pigments.

5.2.1.1 Thickeners

Thickeners are used to enhance the viscosity of formulations. They are explicitly mentioned here, because they are often sources of microbial contamination and are an ideal carbon source for microbes. Different grades of thickeners can be found in the market depending on the necessary CFU-count limit.

Water-based preparations of thickeners must be used within 6 hours after preparation and must not be stored unless a preservation system is in place.

5.3 Storage Requirements

5.3.1 Storage of packed raw materials including packaging materials

- Packed raw materials, including IBCs (totes) as well as packaging materials, must be stored in a dry environment (preferably a warehouse) to minimize possible buildup of microbes on the outside of the packaged material as well as on pallets.
- Unused packaging materials shall not be left uncovered (open).
- Dust development in the warehouse must be minimized to reduce the risk of microbial contamination by airborne bacterial and fungal spores.

Appropriate cleaning procedures for the warehouses must be followed routinely.

5.3.2 Storage of liquids

Since liquids are typically stored in closed containers, the exposure to micro-organisms from external sources is less likely. A critical step is the transfer of raw materials, especially the water-based concentrates, to other containers or to the production vessel open to the atmosphere. Containers with water-based concentrates must remain closed and must only be opened in the staging area. Transferring surplus material from the staging area back to the warehouse must follow the same rule, i.e., immediately closed after use.

Raw materials delivered in bulk / tank containers are considered uncritical for microbial contamination as they are not exposed to the atmosphere. They are handled in closed, dedicated systems and stored typically in tanks.

5.4 Recycling, re-use of equipment and packaging materials

The common basis for all recycled material is the knowledge of their microbiological status. The individual companies each typically handle recycling processes based on their own risk assessments and their corresponding standard operating procedures including handling in the warehouse.

5.4.1 Rinsates, product returns from the market, expired product

- There is a high risk of contamination from recycling of rinsates because of the presence of nutrients, no addition of biocides, the longtime storage and the exposure to the atmosphere during rinsing, providing both a source of contamination and beneficial growth conditions.
- As in other processes, the use of non-registered biocides or registered biocides exceeding registered limits is not allowed.
- A risk assessment must be made before deciding on storage and recycling of any rinsate.
- Similar levels of caution apply when considering recycling product returns from the market, or expired product.

5.4.2 Reprocessing of contaminated products

Whether a product containing microbial growth can be reprocessed depends on various factors. If the product has been irreparably damaged, then it needs to be disposed in a safe manner. A product batch showing microbial growth shall not be blended with a batch free of microorganisms as this is highly likely to result in all the material becoming contaminated.

Reprocessing of product with microbial growth at the plant of an EM can only be carried out after approval by the client and with an agreed reprocessing procedure (most clients will provide this procedure in written form).

5.4.2.1 Gamma irradiation

Irradiation by gamma-rays is a way to reduce or eliminate microbiological contamination. Gamma irradiation has been a common technique applied to medical consumer goods, raw materials for medical or cosmetic applications for many years and is accepted as a standard process of sterilization. This highly energetic irradiation damages irreversibly the microbes – irrespective of the species. The irradiation source is normally Cobalt 60. The irradiation is usually performed by professional specialized companies. These specialized companies offer the irradiation of packed materials. The dose is usually 5 - 12 kGy (kiloGray) for packed products.

It is necessary to check the compatibility of the packaging material with the dose to be applied. PE (polyethylene) bottles can withstand up to 50 kGy, PA (polyamide) usually only up to 20 kGy.

Irradiation can also be done in bulk – in volumes limited to 1 m³. For scaling up to volumes of 1 m³ it is required to increase the dose to ensure full sterilization of the entire bulk.

Usually a two-step approach is chosen: it is recommended to evaluate 2 - 3 different doses to find the best balance between entire elimination of microbial contamination and damaging the product or its packaging. It is necessary to test the product after irradiation to ensure that no damage of the formulation and packaging has occurred. It is also recommended to run storage tests to ensure long term stability of the formulation and that spores and microbes are killed completely.

Currently no limitation to certain formulations types like SL or SC formulations has been experienced, even the application to CS formulations types is possible.

Not all companies offering gamma-irradiation have the approval to store and handle hazardous chemicals. It is recommended to check these possible limitations in advance.

In principle, the gamma irradiation of raw materials like inorganic materials or thickeners is possible. It needs to be verified that the receiving country accepts gamma irradiated products.

5.4.2.2 Heat Treatment

If the formulation allows the use of high temperatures, heat treatment can be applied to sanitize the contaminated product. Conditions of time and temperature have to be evaluated on a case by case basis.

5.4.3 Re-use of potentially contaminated equipment

5.4.3.1 Residual product

A common, often overlooked source for microorganisms is residual product left in the manufacturing equipment (see Figure 7). If microbial growth occurred in the final product and some of that product remains behind in the process equipment, it will seed the next batch with microbes. Since the microbes are already adapted to growing in the product, they will readily grow in the fresh product being made. Furthermore, the microbes may have developed some level of tolerance to the biocide being used to protect the formulation. This process can go on indefinitely unless it will be thoroughly cleaned to remove all of the entire residual product and/or an effective biocide treatment is used.



Fig. 7: A dedicated formulation vessel was not cleaned immediately after the production run, residual product stuck to the walls and stirrer, so biofilm could develop quickly. Although chemical cross contamination is a non-issue in dedicated facilities, this does not apply to the prevention of microbial contamination.

5.4.3.2 Beads for milling

An area containing residual product and/or water that is sometimes overlooked are beads. To prevent residual product and water present in beads from becoming a source of microbes in the next product, several options are available for storage until the next manufacturing run:

- Proper cleaning and complete drying of the beads and storage in a clean, dust free container until the next production run.
- By storing the beads under wet conditions after adding a biocide. Depending on the length of the storage period, a check of potential microbial growth

- before re-use is necessary because the biocide content drops with time.
- Heat-treatment of the beads.

5.4.3.3 Containers and pallets

IBCs and drums that once contained a product with microbial growth can serve as a source of microorganisms for the fresh product that will be stored in this container. Therefore, use new containers. If IBCs or other bulk containers have to be reused, make sure they have been thoroughly cleaned and disinfected to significantly reduce the level of contamination. However, reused plastic containers will always pose a risk of contamination.

In this context, it should be mentioned that dirty/used pallets can often be an important source of microbes and before reusing pallets they should be steamed and thoroughly dried.

5.4.3.4 Flexible Hoses (See also 5.5)

Flexible hoses can be a source of microbial contamination.

After use, all hoses must be cleaned, drained, and dried. Ideally hoses should be hung vertically or in the middle with the open ends pointing to the floor. As soon as the interior of the hose is dry, both ends must be capped to avoid ingress of dust (Fig. 8).

5.4.3.5 Packaging material for end-user product

To avoid contamination of end-user packaging with airborne microorganisms during storage, it is required to keep this packaging material covered and protected against dust as long as possible before placing it on the filling line.

5.5 Plant Design⁷

Manufacturing plants of crop protection products are normally not designed to operate under sterile conditions. However, a preventive approach in plant equipment and design play a major role in the efforts to ensure that products meet microbial contamination limits.

Equipment design for improved cleaning efficiency is not restricted to prevention of chemical cross contamination, but is equally important for preventing microbial growth. This must be considered if new production lines are being designed.

⁷ The following three text books provide more detailed information:

1. G. Hauser, *Hygienische Produktionstechnology* (Wiley-VCH, Weinheim, Germany, 2008).
2. G. Hauser, *Hygienegerechte Apparate und Anlagen* (Wiley-VCH, Weinheim, Germany, 2008).
3. H.L.M. Lelieveld, M.A. Mostert and J. Holah, Eds. *Handbook of hygiene control in the food industry* (CRC Press, 2005; ISBN 10: 0-8493-3439-X).



Fig. 8 Storage of hoses after cleaning and drying. To avoid dust getting into the hose, they must be capped.

Production lines optimized to reduce microbial growth have:

- Clear process design.
- As little piping as possible.
- When possible the use of flexible hoses should be avoided, or at least reduced to a minimum.
- The appropriate type of connections e.g., food industry standard.
- No dead spots or unnecessary connections.
- A piping system with a slope of minimum 4° instead of a horizontal construction to enable complete emptying/self-draining of the system.
- A piping system designed to allow the possibility of easy dismantling.
- Designed to minimize movement of operators, portable equipment, products, IBCs, tools etc. between manufacturing units.
- Designed to minimize dust and aerosol generation.
- Smooth, polished surfaces at least for critical equipment allowing faster, easier and better cleaning (see Fig. 10).
- Rubber hoses, PVC (polyvinylchloride) or other plastic piping should be avoided (see Fig. 11).
- Reduced usage of different diameter piping.

5.6 Housekeeping

The function of housekeeping is to ensure that the manufacturing units and their equipment meet all contamination prevention and hygiene standards on a continuous basis. The following housekeeping rules have to be properly executed:

- Cleaned and dry floors, wall etc. are required. Areas that are not cleaned and dried immediately after a spill allow microbes to develop and formation of biofilm.
- Piping must be drained, cleaned and dried after production. Standing water in piping provides an optimal environment for bacterial growth (Fig. 9).
- Cleaning of the outside of piping should be part of the routine cleaning procedures. Contaminated piping may also be the case in warehouse areas and should not be overlooked. If not done this results in areas with bacterial growth (Fig. 12).

Three housekeeping procedures that need to be mentioned separately because these are easily overlooked:

- Piping and other metallic equipment, which has not been drained after use and has been out of commission for a long period, has to be inspected for corrosion caused by bacteria (pinholes).
- Side glasses must be dismantled and cleaned immediately after the finish of each production run (Fig. 13).
- Filters made of metal must be cleaned on a regular basis (e.g., between batches or campaigns) and, if stored, must be dried. Filters (bags or cartridges) made from plastic shall be replaced. If not cleaned or replaced microorganisms can accumulate and repeatedly inoculate product as it passes through.

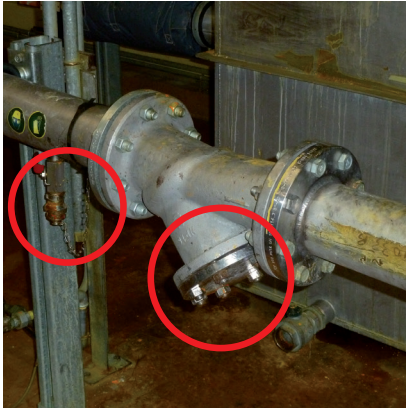


Fig. 9: Two areas where water can be trapped which can result in standing water: the sampling point and the connector. These must be drained to prevent the creation of an environment for optimal microbial growth. Note also bacterial growth on the outside of the piping. Decontamination of the outside of piping must be part of standard cleaning regime.

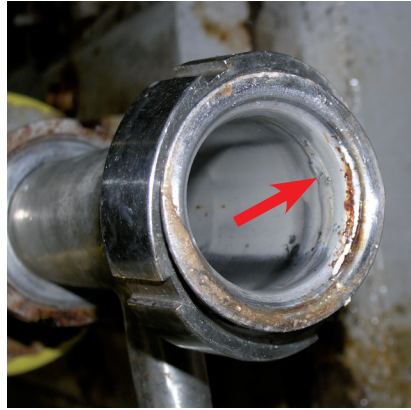


Fig. 10: The need for smooth surfaces also applies to welding. The poor welding of the coupling of a formulation line allowed for standing water and corrosion.



Fig. 11: Sanitization and cleaning plastic piping and rubber sleeves is almost impossible.



Fig. 12 : Microbial growth could readily develop on the outside of piping near the ceiling and thus partially out of sight. In good housekeeping, cleaning the outside of piping will be part of the routine cleaning schedule.

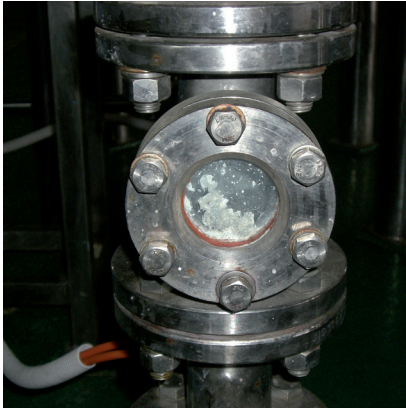


Fig. 13: Accumulation of residue in a sight glass that has not been cleaned after the production run. This residue provides a substrate for microbial growth.

5.7 Personnel

Microorganisms can be introduced in the processing area by operators, maintenance personnel and visitors etc., appropriate personnel hygiene is a must. This includes appropriate coveralls, footwear, gloves etc.

During sampling and carrying out microbial tests in the laboratory aseptic conditions should be applied.

6. Plant Hygiene, Cleaning and Sanitization

To maintain an adequate level of plant hygiene, routine cleaning and sanitization is required. For all the different options listed in this chapter, potential safety and handling issues have to be taken into account.

For the purposes of this booklet:

- A cleaner is defined as a chemical or a blend of chemicals used to remove undesirable soils / stains from a contact surface. The type of cleaner depends on the intended use.
- A sanitizer is a chemical agent that is effective in reducing microbial contamination on product contact surfaces. These chemicals are also referred to as disinfectants.

6.1 Mechanical Cleaning

Mechanical cleaning can involve high pressure water blasting or scrubbing parts/ surfaces with a brush. This may be the only way to remove a deposit. Once biofilms have developed, it is virtually impossible to get totally rid of them, so it would be better to replace those contaminated parts. When cleaning is not effective the only solution, after determination of the “hot spots”, is an exchange of the affected equipment and improved plant design.

A high-pressure cleaner is generally used for larger parts in the formulation plant such as vessels/ tanks, agitators, large piping, and fixed connected charging systems. However, it should be noted that the high-pressure may create aerosols which could spread the microbial contamination. Scrubbing is usually implemented for smaller parts and surfaces.

Pipeline cleaning “pigs” are used for mechanical cleaning of chemical deposits. However, these could also result in mechanical breaking up of biofilm colonies and distribution of “flakes” of biofilm further through the lines where new colonies could be formed (see 10.4). Thus, their use should never be considered as the sole cleaning step for removing biofilms. Additional cleaning steps are necessary to kill and eliminate all residual fragments of the biofilm (see 6.7.1).

An effective cleaning requires the dismantling or opening of the most critical parts of the plant. Furthermore, it may be easier and more effective to replace some parts, such as filters, rather than attempt to clean them.

6.2 Chemical Cleaning

Not all parts and surfaces in a production facility are readily accessible. Therefore, chemical cleaners have to be used for areas that are inaccessible to mechanical cleaning. Typical cleaners include:

- Alkalis.
- Acids.
- Solvents.
- Detergents.

Some cleaners combine alkalis or acids with detergents. The effectiveness of these cleaners can be enhanced by using them at a high temperature ($\geq 70^{\circ}\text{C}$). In addition to cleaning, alkalis or acids can also serve as disinfectants.

Solvents are often used to dissolve chemicals that have poor water solubility. As such, they can be used to remove chemical deposits. Also, solvents are usually effective in killing microorganisms. Therefore, a chemical cleaning program that utilizes a solvent can also be an effective means for disinfection. However, like alkalis or acids, solvents can have potential safety and handling issues that have to be taken into account (e.g., flammability, vapor pressure, inhalation concerns, chemical waste disposal etc.).

6.3 Chemical Disinfectants / Sanitizers

Often mechanical and/or chemical cleaning is not sufficient to eliminate microorganisms from a process. Therefore, a disinfectant / sanitizer may be needed. As mentioned above, alkalis, acids and solvents can serve as disinfectants. However, there are other chemical sanitizers available including the following:

- Sodium hypochlorite.
- Hydrogen peroxide.
- Peracetic acid.
- Quaternary alkylated ammonium chloride mixtures (quats).

The stability of biofilms requires that these products often have to be used at relatively high concentrations. However, due to the stability it is necessary to verify that the sanitization was effective e.g., by means of swab tests.

Sodium hypochlorite / bleach is a well-known disinfectant. It is frequently preferred because of efficacy, cost and handling safety in relation to other disinfectants. However, the construction material should allow the use of chlorine. Sodium hypochlorite can be very corrosive to metal surfaces and reactive with a variety of compounds. It is specifically more corrosive when the concentration

fluctuates during the cleaning. If the hypochlorite is consumed by these compounds, there may not be enough left to kill the microorganisms. Sodium hypochlorite is sold at different concentrations so the amount used will depend on the concentration of the product. The active content of free Cl_2 should be high enough for disinfection of lines (e.g., 0.2 % free Cl_2 is used frequently) and the right pH must be used (non-acidic).

Hydrogen peroxide is another common disinfectant especially for deionized water systems. Typical use contents are 0.5 % active hydrogen peroxide. One advantage of using hydrogen peroxide is that it degrades to water and oxygen, and therefore presents no issues with regard to waste disposal. However, depending on the concentration purchased, safety and handling may be more problematic than with bleach. Also, many bacteria produce an enzyme called catalase, which degrades hydrogen peroxide. This is one reason low levels of peroxide may be ineffective.

Peracetic acid (PAA) (a combination of acetic acid and hydrogen peroxide) is also commonly used as an industrial disinfectant. This combination offers some of the same advantages (i.e., good environmental profile) and disadvantages as peroxide (i.e., potential handling and safety issues). As with the other disinfectants, this product is purchased at a higher content (e.g., 5 % acetic acid, 20 % hydrogen peroxide), and diluted (e.g., 1:100) before use.

Quaternary ammonium compounds (quats) are often used as surface disinfectants. They are effective against bacteria and fungi at contents > 0.5 %. However, the use of quats can cause significant foaming. They are commonly used to wipe down and disinfect surfaces. They are used less often on an industrial scale to disinfect piping, etc. One reason is that the disposal of quats can be difficult as they tend to be detrimental to waste treatment systems.

6.4 Use of Biocides

One way to prevent or reduce microbial growth in the production process is to feed the final product biocide to different parts of the system. This may be particularly helpful if certain parts of the process are highly susceptible to microbial growth. If this is done the biocide must be the same biocide used for final product preservation. Furthermore, the amounts of biocide added to the various process steps must not exceed the registered amount.

Remark:

A treatment of the formulation line by the above procedure is a preventive measure to avoid microbial growth in the final product. It is not designed as a procedure to disinfect production lines to overcome poor plant hygiene.

The following process scheme shows a typical SC line. In this example, biocide is added to the slurry and thickener preparation vessels, and also to the finished product vessel. By adding biocide to the slurry and thickener preparation vessels the whole formulation line is treated with biocide.

The amount of biocide used in the process depends on the quality of the raw materials in terms of microbial growth, and on the conditions of the production line. A typical quantity of biocide used for the process treatment is half of the registered amount of biocide in the formulation. This means, if half of the amount is used for the process, the other half amount is added at the very end of the process just for treating the final product.

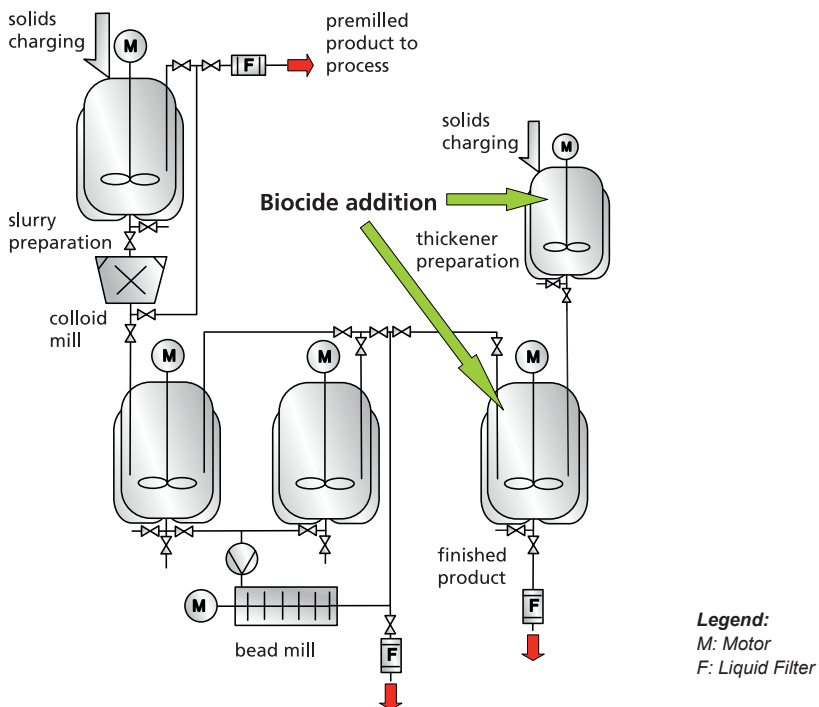


Fig. 14 : Diagram of the manufacture of a SC formulation shows where to add biocide during the process. The last addition of the biocide takes place during the final phase of the process.

6.5 Hot water or Steam

Hot water or steam can be a very effective sanitizer. A major advantage is its ability to penetrate small cracks and crevices.

For hot water, typically temperatures above 80°C and contact times of more than 1 hour are needed. The higher the temperature of the water, the more effective it will be, and the contact time may be reduced. One advantage: hot water/steam is that there is no added chemical (or post-rinses) that must be disposed of in a waste treatment system. However, there is an energy cost, and potential safety issues especially with the use of steam. For treatment with superheated steam, the line must be pressure proof. Please be aware that most formulation lines are not pressure proof.

Most importantly for the sanitization by high temperature is that all parts of the line are heated up to the necessary temperature. Based on the heat transfer through surfaces, the real temperature at a pipe surface or at surfaces of a filter could initially be much lower than the temperature of the heat transfer media (steam or water). Therefore, it must be ensured that the time of treatment at the high temperature is long enough to allow all parts to reach the correct temperature for sanitization. In addition, the time for heating the system up to the correct temperature must be taken into account, and added to the actual sanitization time.

6.6 Drying

Microorganisms need water to grow. To eliminate the opportunity for microbial growth, equipment should be properly drained and dried after rinsing. Evaporation is the simplest and least expensive drying method. Other methods include blow drying, vacuum drying, etc.

This can be particularly important if the plant is not running for a period of time. When there are areas in the equipment where water can accumulate and cannot be drained, some residual disinfectant or biocide must be added to prevent microbial growth. This preserved water will need to be flushed out with clean water before the next production run.

6.7 Treatment Strategy

The type of cleaning and sanitization that a plant utilizes, and its frequency, will depend on the severity of the microbial problem. Every plant is different, and may require a different treatment strategy. Furthermore, other factors (e.g., waste disposal limitations) may affect the decision process on what to use, and how to use it.

Cleaning must always precede sanitization except when using a combined cleaner/sanitizer agent.

Relying solely on one cleaning agent or one disinfectant is typically not as effective as using a combination. This combination may involve a sequence of two cleaning agents with disinfectant activity (alkali and acid), or a cleaning agent followed by a disinfectant. For example, combinations of sodium hydroxide followed by sodium hypochlorite can be very effective.

An example of an effective cleaning strategy is described below:

1. Mechanical cleaning to remove visible deposits and residual product. (e.g., high pressure washing of vessels). This should be drained before the next step.
2. Chemical cleaning especially for areas that cannot be readily accessed (e.g., pipes, pumps, etc.). This may involve alkalis, acids, solvents or detergents. After cleaning, drain the residual cleaning agent.
3. Rinse the areas affected by the cleaning agent with clean water.
4. If needed, add a chemical disinfectant (e.g., sodium hypochlorite) and circulate to critical areas. Drain the disinfectant.
5. Rinse with clean water. Several rinses and drains are likely to be needed to remove residual disinfectant, and prevent product contamination.
6. If the line is not used immediately afterwards, the line must be kept dry.

Hot water or steam may be substituted in place of the chemical cleaning/disinfectant agents in steps 4 and 5.

6.7.1 Strategy required when biofilm is present.

Incomplete removal of biofilm during a disinfection will enable the microorganisms to rebuild a biofilm or even form new biofilms. To reduce the chances of continuous infestation, it is critical to have an effective cleaning strategy.

Since microbes living in biofilm are hard to kill and biofilm can grow in places in the equipment that are not easily accessible, this presents a big challenge. The required strategy must be product / equipment specific, and to find the optimum cleaning procedure local trials are necessary. Factors to consider in the design of this strategy are the level of infestation, plant design and material characteristics of the equipment, and the possibility of dismantling, restrictions of the disposal of waste water etc.

It is not possible to provide a general set of recommendations that cover all options, however, the following sequence of cleaning steps may help to develop a possible scenario:

1. Mechanical cleaning (e.g., brushing or use of “Pigs”) and/or high pressure hot water,
2. Alkali with a pH > 12 and > 1 hour treatment time,
3. Drain and wash the equipment down with water,
4. Circulate sodium hypochlorite solution (e.g., 0.2 % to 0.5 % of “free” chlorine) for > 1 hour and drain,
5. Rinse the equipment with water and drain,
6. Run a microbiological test to determine / measure critical control points (swap testing),
7. Keep the equipment dry, when production will not follow immediately after disinfection.

6.8 Organizational measures

Besides all the different types of cleaning and sanitization processes, it is of particular importance to also have the necessary organizational measures in place to achieve an adequate level of plant hygiene. These include, but are not limited to, proper production planning, effective cleaning and operating procedures, regular monitoring and adequate training for all employees.

7. Microbiological Test Methods

There are many methods available for testing microbial growth in a chemical product. When choosing a method various factors must be considered:

- Time-frame for availability results
- Frequency of testing
- Required precision
- Qualitative or quantitative results

In addition, the skill level of the person(s) performing the tests and the availability of suitable laboratory equipment are very important considerations for obtaining meaningful results.

There are three general types of microbiological test methods based on their function:

a. Detection of the presence or absence of microorganisms

Presence / absence tests are designed to detect the presence of any organism in a sample or to detect a specific microbial species. Simple and rapid methods include the dipstick, BioLumix®⁸ or BacTrac®⁹ which will give semi-quantitative results.

b. Enumeration of microorganisms

Enumeration tests determine how many microorganisms are present in a sample. The ability to enumerate microorganisms is influenced by many factors, including condition and duration of incubation, incubation temperature and whether the organism is stressed or shocked. There are two overall methods to choose from: direct inoculation (e.g., Standard Plate Count); or inoculation following membrane filtration.

c. Identification of microorganisms

Identifying the microorganisms present in the test sample can help to determine the source of the contamination:

- *Pseudomonas* species may indicate a contaminated water source,
- *Staphylococcus* species point at a personal hygiene issue,
- *Bacillus*, yeasts and molds can point to facility issues, e.g., air contamination.
- The raw material may be a source of any of these

In case identification is necessary selective agar media, commercial test kits, or DNA sequencing can be used.

The product tested must be compatible with the growth media (does not inhibit growth) and that the micro-organisms of interest can grow on the media being used.

⁸ BioLumix is a registered trademark of Neogen.

⁹ BacTrac is a registered trademark of SY-LAB Geräte GmbH.

7.1 Standard Plate Count Method

The most common way to test and enumerate the presence of bacteria and fungi is to spread an aliquot (0.1 ml) of the sample onto an agar plate. Various types of agar plates are available for growing different types of microbes: bacteria, fungi, yeasts or molds.

When present, each of the individual microorganisms will form colonies which can be counted after a suitable incubation period.

For testing microbial contamination of chemical products, usually non-selective media are chosen capable of growing a broad range of microorganisms (e.g., Plate Count Agar, Tryptic Soy Agar). Typical incubation conditions for bacteria are 48 - 72 h at 30°C, and for fungi 48 -120 h at 25°C. After this incubation period the number of colonies are counted. The results are expressed as a ratio: number of Colony Forming Units/ml or g, ([CFU/ml] or [CFU/g]).

Prior to use, agar plates normally need to be stored at temperatures lower than those used for incubation, while shelf life requirements specified by the supplier must be followed.

For SC or SE dispersions it is common practice to apply 1 ml solution of a 1 % (v/v) dilution to allow the formation of a transparent layer. Under these circumstances the reliable counting of the CFUs is possible despite the presence of particles in the formulation.

Highly viscous or solid samples (usually raw materials) could be dispersed in sterilized water, usually starting at 1 to 5 %. Normally, a 0.1 ml aliquot is applied onto the center of the agar plate; the liquid is then evenly distributed with a disposable loop. To ensure the sample is representative it is important that a part of the solid is also transferred onto the plate, not just the supernatant.

Commercial agar plates can take up to 1 ml solution. This ensures a homogeneous distribution of the potentially contaminated liquid over the entire plate allowing reproducible counting of CFU/ml.

If the concentration of bacteria and/or fungi is high, and an accurate count is necessary then the sample must be diluted. Typically a sterile buffer/water is used to make a series of 1:10 dilutions and each dilution is spread onto an agar plate. After the incubation, any agar plate that has a colony number between 30 - 300 is counted. The number of colony counts is multiplied by the dilution factor to determine the concentration of microorganisms in the original sample. This is reported as CFU per ml or g of sample.

If only qualitative or semi quantitative results are required, a sterile swab can be inserted into the sample and then swabbed onto an agar plate.

Based on the number of colonies (Fig. 15) detected, the level of microbial growth can be determined as described in ASTM D2574-06:

0 = No bacterial recovery

1 = Trace contamination (1 to 9 colonies/ml or g)

2 = Light contamination (10 to 99 colonies/ml or g)

3 = Moderate contamination (≥ 100 distinct colonies/ml or g)

4 = Heavy contamination (continuous smear of growth, colonies have grown together and are indistinguishable).

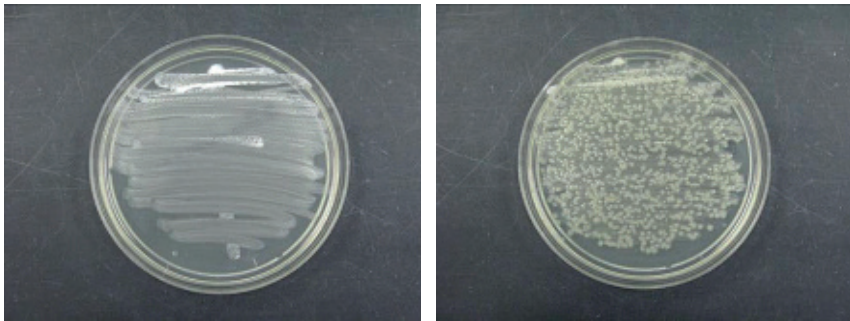


Fig. 15: Example of 0 =no bacterial recovery and 3 = Moderate contamination (≥ 100 distinct colonies).

7.2 3M™ Petrifilm™¹⁰

Petrifilm is a dehydrated, sterile ready-to-use film based medium. It is used for enumeration of microbes. Petrifilm has a number of advantages over traditional agar plates. For example, it is ready to use, has a long shelf life when stored correctly, requires very little space and handling is convenient. The Aerobic count films have an indicator which aids visualization of colonies by staining them red. The recommended counting range for the aerobic count film is between 10 to 300 CFUs. However, the counting range of Petrifilms can be easily estimated up to 500 colonies by means of the 1 cm grid pattern printed onto each film. It is advised to count a minimum of two squares, one with a high density of colonies, one with a lower density of colonies. When these counts are added together and multiplied by 10 this will give an accurate estimation for the total 20 cm² growth area. To read Petrifilm automatically, commercial readers are available.

Swab samples can also be plated out onto. Swabs need to be rinsed into sterile diluent or commercially available nutrition broths and then plated the same way

¹⁰ 3M, Petrifilm, and Clean-Trace are trademarks of the 3M Company.

as a sample. Swabs cannot be directly applied to Petrifilms as water is needed to rehydrate the films.

For detailed instructions refer to the supplier.

7.3 Membrane filtration

This method may be used for measuring microorganisms in water or diluted formulations e.g., SLs with a low concentration of microbes. Commercially available canisters with the sterile membrane filter are placed on a suction pump. The canister is filled with a defined volume (e.g., 100 ml) and attached to the pump. The membrane will be removed and placed on a Petrifilm or an agar plate for incubation followed by enumeration.

7.4 Dip Slides

Dip slides contain agar media on a plastic support. Dip slides can be purchased with various types of media for growing different types of microorganisms. Some contain bacterial growth media on one side and fungal growth media on the other.

Dip slides are supplied in a sterile container and are ready for use. The dip slide is taken out of its container, dipped into the sample solution for several seconds, removed, and placed back into the sterile container. The dip slide is then incubated for 48 – 72 h at 30°C. After the incubation period, the number of colonies on the dip slide is compared to a reference chart to determine the approximate concentration of bacteria and/or fungi in the sample. Dip slides are not as accurate or sensitive as plate counts or using Petrifilms.

7.5 Microbial Activity Assays

The tests mentioned in 7.1 to 7.4 rely on counting microbial colonies that develop on an agar plate, dip slide or Petrifilm. An alternative way to determine the presence or absence of microbes is to monitor microbial activity. Basically, a product sample is put into a vial containing a medium that allows growth of the microbes. This growth can be measured by monitoring changes in the media (e.g., CO₂ production, lower pH, turbidity, luminescence, conductivity, etc.).

The advantage of methods based on these principles is that they are considerably faster than those described earlier.

Examples of these methods are discussed in 7.5.1. and 7.5.2.

7.5.1 BioLumix

BioLumix is an automated system for the detection of aerobic microorganisms. By using an optical sensor, this system measures changes in the color or fluorescence of an indicator which are caused by release of carbon dioxide (CO_2), the universal metabolite of aerobic microorganisms.

The CO_2 passes through the membrane at the bottom of the disposable vial and reacts with the indicator in the reading zone (Fig. 16). Only gases can pass through this membrane, not microorganisms, liquids or particles

The availability of a range of media makes it possible to use this method for the detection of a range of microorganisms: total aerobic bacteria, specific bacterial species, yeasts, or molds. All the types of media for bacteria provide results within 18 to 22 hours, however, the ones for yeasts and molds require 48 hours to give results.

The BioLumix technology can be used for formulations with high pigment loading. These formulations could not be checked for microbiological contamination when using the plate or Petrifilm methods.

The initial dark color of the indicator in the reading zone of the vial changes to orange-yellow due to CO_2 production caused by microbial metabolism. The broth in the top part of the vial (= the incubation zone) does not contain any indicators.

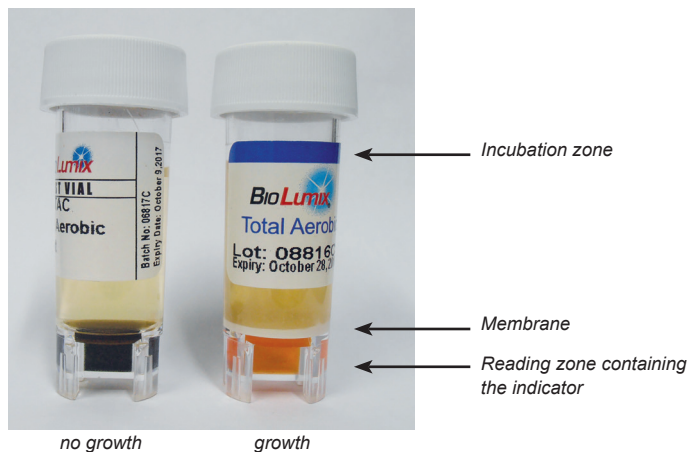


Fig. 16: BioLumix: system for detecting microbial growth

illustrated in a hypothetical example for three different samples: 1. highly contaminated, 2. low infestation and 3. sterile water (See Fig. 17).

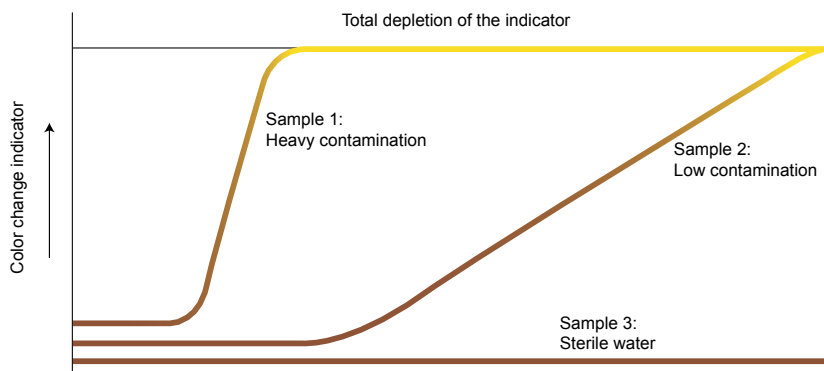


Fig. 17: Principle BioLumix method.

The rate of microbial metabolism is a direct measure of the contamination level of a sample (=CFU-count). The speed with which the intensity of the color of the indicator changes (see Fig. 16) indicates the level of contamination.

The speed of the color change indicates the rate of the microbial growth (metabolism). The earlier the color starts to change the higher the microbiological contamination. Ultimately, this results in a straight, horizontal upper line due to complete consumption of the available indicator in the detection cell. When this stage has been reached, it is no longer possible to measure further microbial growth.

Sample preparation is easy by direct addition of the sample into the vial: up to 1 ml of liquids or 100 mg of solids. The sample size depends on the expected level of microbial contamination.

A limitation of this method is that it is not quantitative, i.e., an absolute number of CFUs cannot be determined; to obtain accurate CFU numbers, a cross validation with plate counts will be necessary.

It is recommended to check sensitivity of the microorganisms to incubation temperatures; microbes adapted to cold media might not grow with sufficient speed at elevated temperature to generate a change in baseline.

Besides the BioLumix system, there are other suppliers offering instrumentation based on the same principle.

7.5.2 BacTrac 4300®

BacTrac is an automated system that monitors the current resistant of the microbial metabolism activities using pin electrodes. This method can be used for the detection of aerobic and anaerobic bacteria, yeast and molds, etc.

7.6 ATP (adenosine triphosphate) Method

The methods listed above all require an incubation period (up to several days) before the results are available. There may be situations where a more rapid turnaround time is needed. One possible way is to utilize an ATP based test. For clear water or water based solutions, results may be available within minutes. For all samples, which need sample preparation, a longer time is required.

ATP is present in all living cells. The amount of ATP in a sample can be used as an indicator of the amount of microbial growth. Dead, non-viable microbial cells can also contain ATP and this would be measured by the test. In addition to microbial ATP, the presence of non-microbial ATP will also be detected.

The test method uses a chemical reaction with ATP. The reaction emits light which is measured typically by a Photo Multiplier Tube (PMT) or photodiode (see Fig. 18). The emitted light is directly proportional to the amount of ATP present, as long as the system stays in its linear range.

Since the test method involves the detection of light, the transparency of the sample has a major impact on the efficacy of the test by absorbing the light produced during the reaction. Samples that are opaque, SC, SE etc. are problematic. Some chemicals may disrupt the chemical reaction and reduce the



Fig. 18: Example of an ATP testing device – the 3M™ Clean-Trace™ luminometer.

light output. Positive control tests are available to check for any disruption of the test reaction. The design of self-contained tests helps to overcome the risks of contamination, but care should be taken to avoid touching the sample device during testing.

7.7 Swab testing

To check whether the disinfection and cleaning of the equipment have been effective, swab testing is an important tool. It is a direct test for microbiological contamination of surfaces inside and outside of an equipment rather than an analysis of the rinse water. The potential downside of the swab test is that only parts of the equipment or of a production line are sampled. Therefore, the surfaces selected for the swab test must be representative for the part of the line to be tested to avoid either false positive or false negative results.

To successfully use swab tests, all steps must be performed in a hygienic, sterile manner to avoid potential contamination.

The surface tested for microbiological contamination must be free of residuals of disinfectants because it will be impossible to detect microbial growth under these circumstances.

Various suppliers are offering kits for swab testing to allow convenient handling. The kit usually contains its own sterile culture broth reservoir. The swab is wetted with this culture broth just before use. The supplier's instructions should be followed in detail, including storage and shelf life.

Normally, the swab should be swiped backwards and forwards across the test surface (typically 10 cm x 10 cm) rotating the swab. This motion should be repeated until all sides of the swab have been in contact with the area to be tested for microbial contamination. After completion, the swab must be placed in the sterile broth tube and the lid closed. Swab solutions must be plated within a defined period – following the supplier's recommendations. The plates must be incubated for 72 hours and then read for growth.

Contact plates, which are offered commercially, can also be used for flat surfaces. After use the tested surface must be cleaned to remove any remaining agar.

8. Biocides

A biocide is commonly added to aqueous formulations to preserve the product until end of shelf life when stored under recommended conditions. The biocide should prevent microbial growth, but it is not the function of a biocide to overcome poor plant hygiene. The total amount of biocide added must not exceed the registration limit, even if the biocide is consumed during manufacturing.

8.1 Type and chemistry of biocides

Biocide actives used for the prevention of microbial growth in chemical products can be separated into two major modes of action: the electrophilic and the membrane active substances. Some kill microbes quickly e.g., Bronopol. Others act more slowly over weeks and months e.g., BIT, or years, e.g., MIT.

Electrophilic Substances	Membrane Active Substances
Aldehydes <ul style="list-style-type: none">- Formaldehyde ¹⁾- Formaldehyde releasing compounds ¹⁾- Glutaraldehyde- Glyoxal	Alcohols <ul style="list-style-type: none">- Benzalcohols- Phenoxyalcohols
Activated Halogen Compounds <ul style="list-style-type: none">- Bronopol- Chloracetamide- Dibromodicyanobutane- Dibromonitripropionamide	Weakly Lipophilic Acids <ul style="list-style-type: none">- Benzoic acid- Sorbic acid
Isothiazolinones <ul style="list-style-type: none">- Methylisothiazolinone (MIT)- Chloromethylisothiazolinone (CMIT)- Benzisothiazolinone (BIT)	Cation Active Substances <ul style="list-style-type: none">- Quarternary Ammonia compounds- Guanidine

1) Formaldehyde and formaldehyde releasing compounds have been used extensively in the past. However, regulatory constraints now preclude their use in agrochemical products in some regions.

The biocide actives that are allowed in agrichemical products is a small subset of the list above, and will vary depending on the country or region. The most commonly used actives are the isothiazolinones. Some biocide products may have a single active (e.g., BIT), others may contain a combination of actives (e.g., CMIT and MIT). Combinations of biocides often show synergistic effects like a MIT/BIT mixture with significantly increased preservation possibilities compared to same amounts of the single biocide.

Biocide Efficacy Testing

To identify the most effective and suitable biocide from those that are registrable, a challenge test is commonly used. Challenge tests should be conducted in the commercial packaging of a product to consider physical and/or chemical impact of the packaging material like adsorption or oxygen penetration. Typically, aliquots of the formulated product with different amounts of the biocides are evaluated. The product samples are spiked with a mixture of microorganisms capable of growing in the product and compared to a product sample without biocide present. Ideally and when available these microbes should come from the product itself and/or from the manufacturing site(s) where the product will be made. If there is some product available that already has microbes present, this can also be used as part of the challenge. Usually, this is the best type of challenge inoculum, because the microbes are already conditioned to growing in the product.

Directly after spiking, the product samples are tested for the presence/absence of microbes. Following this, repeated spikes (typically 3 to 4) of the same product are conducted over a period of time (e.g., 1 – 2 months). After each challenge, the samples are tested for microbial growth.

Biocide stability

The required biocide stability must be considered when choosing a biocide. At the very least, a biocide should control the microorganisms in a product until the end of the shelf life. The length of time that a product will be protected against microbial growth will depend on which microbial pressures the product will be exposed to, the general storage condition, as well as, the stability of the biocide. Biocide stability can also be affected by a variety of other factors including product chemistry, high levels of amines, reducing and oxidizing agents, pH, oxygen, adsorption on active surfaces and absorption in packaging material, and storage temperature (Fig. 19).

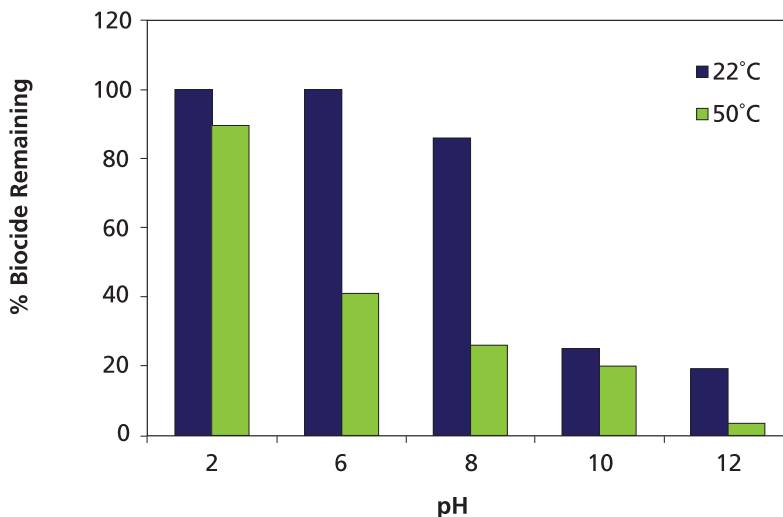


Fig. 19: Effects of pH and temperature on CMIT stability in water after 3 months.

Bio-availability

Another important aspect is the bio-availability of biocides. For example, benzisothiazolinone (BIT) and methylisothiazolinone (MIT) are sensitive to polar molecules. They may adsorb at the surface of such particles (e.g., dispersed active ingredients or even fillers like highly porous silica derivatives) and will thus no longer be available as a biocide.

The model diagram (Fig. 20) below shows how a biocide concentration may change over time. After a biocide is added, a portion may be adsorbed or interact with the formulation matrix; another part might be used up in killing existing microorganisms in the product. Only the residual amount is available for the long term preservation of the product. This aspect is important and should be considered in product development.

Tolerance

Microorganisms can adapt to the biocide being used to control them. This is referred to as biocide tolerance. As this develops, a concentration of biocide that

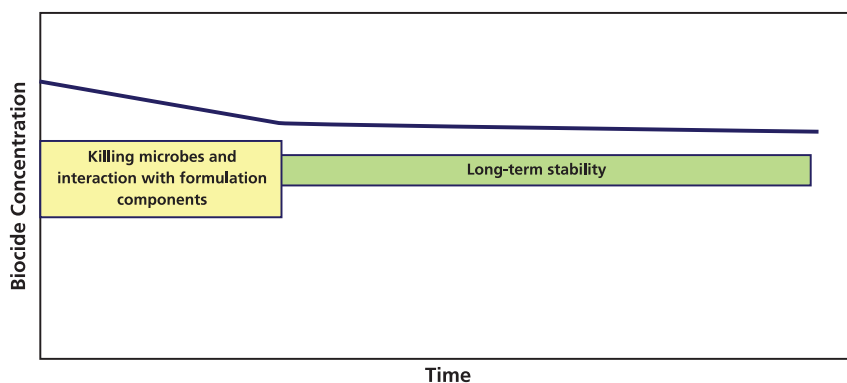


Fig. 20: Effect of time on the biocide concentration in formulations.

was once effective may no longer be effective in controlling the microorganisms. This is often a problem when *Pseudomonas* bacteria are present, and the biocide contains only one active ingredient. If the biocide contains multiple actives in sufficient concentrations, biocide tolerance is much less likely to occur.

8.2 Regulatory requirements

The use of biocidal products in crop protection products is strictly regulated in most countries. Products containing one or more biologically active substances, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any organism by chemical or biological means, require authorization. Legislation exists for example:

- Biocidal Products Regulation 528/2012 in the EU.
- Biocides in the USA are regulated under the Federal Insecticide, Fungicide, and Rodenticide ACT (FIFRA), which requires the Environmental Protection Agency (EPA) also to register formulations for distribution and sales including type of biocide and contents (EPA 40CFR, part 158W, sub-part W [158W], effective July 8, 2013).

9. Microbiological Contamination Risk Assessment

To ensure no microbial contamination will take place during the manufacture of any new water-based crop protection products, it is essential to thoroughly assess the site and process for potential microbial contamination. This should take place irrespective of where the production is planned. This risk assessment helps to determine the microbiological prevention strategies required. The questions listed in the Appendix (pages 68-82) may serve as a helpful starting point for this assessment.

The elements that need to be included in the risk assessment:

- **Limits for microbial contamination**

It is the responsibility of each individual company to set the limits for microbial counts required to avoid unacceptable contamination, except for regulated ones. Limits should be set as needed for:

- Raw materials.
- Water.
- Finished product
- Critical control points.

- **Infrastructure and Plant Design**

Production sites and equipment have not necessarily been designed using the principles of microbial contamination prevention; so for a risk assessment it is important to identify areas especially prone to microbial growth – critical control points, the so-called hot spots. Section 5.5 – Plant Design, discusses areas prone to microbial growth.

It is necessary to determine the contamination levels at these critical control points in the equipment to evaluate whether the levels are below the critical threshold. In case the limit is exceeded or trends are observed, corrective actions must be taken.

- **Plant Hygiene (refer to Chapter 6 - housekeeping).**

- **Training and level of awareness.**

A sound training and awareness program increases job knowledge and helps to motivate employees. Each manufacturer shall establish training needs and ensure that all personnel are trained to adequately perform their assigned task. Training should be periodically reinforced and documented.

- **Procedures and documentation**

Manufacturing activities, including laboratory testing, must be properly documented, and should have procedures in place to help ensure consistent process and practices. Companies should have documented procedures that describe how the product is made, tested, stored and released, how to clean the equipment, dispose of waste and how to prevent microbial cross-contaminations.

10. Incident Case Histories and learning Experiences

The key to successful prevention and control of microbial contamination in crop protection products is the implementation of the best practices and standard operation procedures described in this booklet. The learning experiences gained from actual incidents form the basis for these “Guidelines”, rather than being developed only from theory. Some examples of real incidents will make it easier to understand the rationale for the reasons for the “Guidelines”. Also, when involved in training staff on the prevention and control of microbial contamination, presenting the case histories in this booklet will help to capture the attention and interest of the audience because it is easier to relate and understand real life experiences.

10.1 Case History 1: Poor quality of Process Water (see 5.1.1)

A suspension concentrate (SC) developed an unpleasant odor and showed precipitation during storage in the warehouse prior to shipping. This was attributed to microbial growth.

For quality reasons all batches were rejected and had to be incinerated at considerable costs; they could not be disposed of as liquid waste.

By not being able to supply product in time, the reputation of the product was damaged for the coming growing seasons.

The root cause investigation showed:

- The process water was sourced from a water purification plant that utilized tanks for demineralized water. These tanks were not periodically disinfected.
- This water was transported to the formulation plant and stored in rubber coated tank wagons, sometimes stored under direct sunlight, also during hot summer days.
- A plant inspection showed microbial infestation (including biofilms!) of the water storage tanks and parts of the actual formulation plant, especially large surface areas – filter devices, heat exchangers.
- A routine cleaning schedule was not part of the housekeeping procedures.

What can be learned from this case history?

- Periodical “microbial” cleaning of the purification plant, storage tanks and the formulation plant should be done following a fixed routine schedule.
- A further recommendation made after the plant inspection: Attention should also be paid to parts that are difficult to clean: pumps, ball valves, sampling points, seals between flanges etc.

10.2 Case History 2: Poor quality of Water used for equipment cleaning (see 5.1.2)

Several hundred thousand liters of a SC had to be withdrawn from the supply chain and reprocessed due to microbiological contamination.

The root cause investigation showed:

- In a cost saving program one of the decisions was to reduce the waste water disposal costs by recycling all rinse water.
- The water used for equipment cleaning (rinse water) was recycled. The rinse water storage tanks were not sufficiently treated and served as the ideal environment for microbial growth.
- The microbes in the contaminated rinse water re-infested the equipment and provided the bacteria in the next product.

The conclusion from the root cause investigation was to stop recycling rinse water immediately. Further analysis showed that recycling would only be feasible by building and operating an expensive water treatment unit on site.

What can be learned from this case history?

- Recycling of rinse water should be avoided, but if adopted:
 - o The quality criteria used for process water are also applicable to rinse water to help prevent microbial contamination in the following product.
 - o Adequate treatment of spent rinse water needs to be in place.

10.3 Case History 3: Bad Housekeeping and inadequate sanitization procedures (see 5.4.2, 5.6 and 6)

Microbial growth was observed in several water-based formulations. These were not isolated events, but frequent recurrences. This required a number of product recalls and badly impacted the confidence of the end-users in all the water-based formulations of this company.

The root cause investigation showed:

- In a plant inspection it was discovered that parts of the formulation line tested positive for microbial growth.
- The microbiological testing methods used were inappropriate which automatically showed negative results – no microbes present in the equipment.
- Non-used parts of the production line were not cleaned immediately after the production run, were still connected by ball valves and remained filled with water for months.

- Inspection of the operating procedures showed:
 - o Incorrect manufacturing practices.
 - o Bad housekeeping procedures.
 - o Inadequate disinfection guidelines.

What can be learned from this case history?

- New microbial testing methods need to be introduced and validated.
- Review, develop and validate new production and housekeeping practices.
- After completion of each production run, the equipment should be cleaned immediately followed by drying.
- Improve the training programs and their frequency.
- Need to raise awareness of the vital importance of microbiological contamination prevention across the organization.

These events resulted in a strong, management driven remedial program based on the learning experiences from the root cause investigation.

10.4 Case History 4: Cleaning when biofilms are present (see 4.3)

Microbial growth was detected in several products formulated in a SC-plant, but disappeared after an in-depth cleaning campaign. However, the problem came back after a couple of weeks. The contaminated product had to be irradiated.

The root cause investigation showed:

- Several (hard to clean) “Hot Spots” were identified from which micro-organisms originated.
- The entire plant was chemically disinfected which initially stopped microbial growth. However, after one month the problems came back.
- Biofilms were identified as the root cause.
- The cleaning and sanitization procedures were not appropriate to remove the biofilms.

What can be learned from this case history?

- Identification of “Hot Spots” is very important, because these require extra attention in the cleaning procedures.
- Microbes inside biofilm are protected by being less accessible to disinfectants. An appropriate chemical for dealing with biofilm, often at a high concentration, is required.
- In case biofilms could not be removed by cleaning and sanitization different measures must be used, e.g., mechanical means or hot water treatments.

10.5 Case History 5: Recycling rinsates (see 5.4)

To reduce water consumption, wasting AI, and disposal costs of rinse (waste) water, the first 600 liters of rinsate were collected after the campaign of each water-based formulation and stored in IBCs till the next production run of that particular formulation. A number of the stored IBCs started to give off a strong, very unpleasant odor before they could be recycled. A nearby farmer alerted the fire department because he feared there was a gas leak. This resulted in two fire engines being rushed to the plant. This incident damaged the otherwise excellent goodwill of the manufacturer.

The root cause investigation showed:

- The IBCs with rinse water were stored in a warehouse during the summer.
- Temperatures during the storage period were as high as 35°C for prolonged periods (creating optimal growing conditions for many microbes: high moisture and favorable temperatures).
- Further investigation showed that in all cases the rinse water showed a very high bacteria count (CFU-count).
- The stored rinse water had to be discarded.

What can be learned from this case history?

- Aqueous rinsates, especially when stored over long periods between campaigns are not suitable for recycling into new product.

10.6 Case History 6: Contaminated Raw materials (see 5.2.1.1)

A shipment of 60,000 liters of an SC formulation was stopped by customs inspectors at the border. The product was out of specification and importation was blocked. The product showed a loss of viscosity and phase separation (see fig. 1). At considerable costs the product had to be shipped back to the country of origin followed by disposal as hazardous waste.

The root cause investigation showed:

- Microbial degradation of the thickener caused the formulation to fail.
- Poor hygiene combined with the use of poor quality thickener were responsible for a high microbial count of the returned product.
- In addition, the thickener was prepared weeks before the production run and stored for 4-5 weeks under unfavorable environmental conditions.
- The product release procedure did not include checks for microbial contamination.

What can be learned from this case history?

- Thickeners are a known source of microbial contamination and careful selection of the correct grade of thickener with known CFU-count is required (see 5.2.1.1). A high quality grade of thickener is recommended.
- The initial quality of the formulants is very important as it is a major contributing factor of the potential microbial contamination level in the finished product.
- Thickeners should be prepared just prior to manufacture of the formulation and should not be stored longer than six hours.
- Water-based formulations should not be released without checking them for microbial contamination (CFU-count).
- Implementing updated housekeeping and release procedures proved to give an effective solution of the contamination issue.

GLOSSARY

ASTM	American Society for Testing and Materials
Aerobic Organism	Organisms living in the presence of free oxygen; for their metabolism, molecular oxygen is a requirement.
AI	Active Ingredient.
Anaerobic Organism	Organisms which metabolic processes do not require oxygen and can survive in an oxygen free atmosphere or in a greatly reduced concentration of free oxygen.
Aseptic	Very low level of microorganisms.
Bacteria (Bacterium - singular)	Small prokaryotic, single-cell micro-organisms typically 1 µm to 5 µm in length / diameter (not visible with the naked eye).
Big Bag	Big bag is the commonly used term for a FIBC (= Flexible Intermediate Bulk Container). Also called bulk bag. Used for storing and transporting dry bulk goods like powders, granules, pellets etc.
Binary Fission	The process of each single cell producing two identical cells. The process of multiplication in bacteria.
Biocide	A biocide is defined in the context of this booklet as a chemical used to destroy or have a controlling effect on any microorganism by chemical or biological means.
Biocide Tolerance	A condition in which microorganisms are no longer killed or inhibited by a biocide.
Biofilm	A group of microorganisms with the cells sticking to each other attached to surfaces, e.g., in processing equipment, piping etc. The cells are embedded within a self-produced matrix consisting of extracellular DNA, polysaccharides, enzymes and other proteins, and water. Cells inside a biofilm are much harder to kill than those of the same species living as single cells in an aqueous medium.
CFU	Colony Forming Unit – individual or clumps of cells / spores that grow into a colony on a solid media surface (e.g., agar plates, Petrifilm or dip slides).

Cleaner	A chemical or a blend of chemicals used to remove undesirable soils / stains from a contact surface. These chemicals can be detergents, solvents, etc.
Client	Company contracting the manufacturing of a product (Als, raw materials etc.) with an external manufacturer.
Contamination, Microbial	The undesired introduction of microorganisms in finished products, intermediates, raw materials including packaging material, production equipment and production areas.
Contamination Prevention	Any measure, be it organizational or technical, to prevent the occurrence of contamination.
CropLife International	Global Association of Multinational, Research-based Crop Protection Companies and its regional industry associations
Dip Slides	A test method for the presence of microorganisms in liquids. The dip slide test consists of a sterile culture medium on a plastic support that is dipped into the liquid to be sampled.
Disinfectant (Sanitizer)	An agent, normally a chemical (or a mixture), applied to surfaces to destroy microorganisms. See also: Sanitization.
DNA	Deoxyribo Nucleic Acid. DNA is a macromolecule containing the genetic information / instructions for growth, development, functioning and reproduction of all known living organisms.
Endospores	Endospores are formed by certain bacterial species when the environmental conditions become unfavorable (e.g., desiccation, depletion of nutrients etc.). The Endospore phase will allow survival for very long periods.
Enzymes	Proteins that perform biochemical functions, e.g., the break-down of macromolecules such as polysaccharides to mono- or disaccharides.
Eukaryotic Cells	Cells in which the chromosomes are “enclosed” in a membrane, the nucleus. Eukaryotic cells also have membrane bound organelles such as mitochondria and the Golgi apparatus. In contrast to bacterial cells, all animal, plant, and fungal cells fall in this category.

External Manufacturer (EM)	A company manufacturing products for crop protection companies on a contractual basis. Synonymous with: contract manufacturer, contractor, toll manufacturer ("toller").
Fermentation	A metabolic process that converts sugars or other organic molecules to organic acids, gases (CO ₂), or alcohol. Fermentation occurs both in bacteria, yeasts and molds.
Flexi hose	Flexible hose, used for transfer of materials when no fixed piping is installed; requires special attention in microbial contamination prevention.
Flowable concentrate (SC)	Flowables. See Suspension Concentrate
Flowable concentrate for seed treatment (FS)	A stable suspension for application to the seed, either directly or after dilution
Formulant	Any substance other than technical active ingredients intentionally incorporated in a formulation
Formulation	A preparation of active ingredient(s), and the "inert" chemicals required to form a stable product allowing the application of the AI(s) either directly or after dilution.
Fungus (plural: Fungi)	A fungus is any member of the group of eukaryotic organisms that includes unicellular microorganisms such as yeasts and molds, as well as multicellular fungi which can be visible with the naked eye.
Gel for direct application (GD)	GD is the designation for a gel-like preparation, intended to be applied undiluted. A gel for direct application consists of one or more active ingredients, a structuring agent and other formulants if appropriate.
"Hot Spots"	Typically hard to clean areas in manufacturing equipment that are prone to harbor microbial growth.
Housekeeping	Process ensuring the manufacturing units and their equipment meet all contamination prevention and hygiene standards on a continuous basis.
Hygiene practices	The set of practices of keeping a manufacturing plant or process clean, esp. free of undesirable micro-organisms.

Hyphae	The tubular multicellular network of fungi.
IBC	Intermediate Bulk Container; movable container for liquids or solids.
ISO, Isotainer, ISO tank container	International Standards Organization standardized container for liquids, which can be transported by road, rail or ship. Typical volumes are between 17,500 and 32,000 liters.
Incubation period	Time required for single microbes to develop into colonies with a size that allows counting with the naked eye.
Infestation	The presence of a large number of microorganisms in a product, raw materials, manufacturing facilities etc. at a level that can cause microbial contamination and damage.
Inoculum	The sample (e.g., 1.0 ml) of the material that is tested for the presence of microorganisms.
Microbiology	The study of microscopically small organisms, which can be unicellular (single cell), multicellular (cell colony), or acellular (lacking cells, e.g., viruses).
Microbiological pesticides	Microbiological (Microbial) pesticides consist of bacteria, fungi, protozoa or viruses (and sometimes include the metabolites that bacteria or fungi produce) used for crop protection.
Micrometer, micron (µm)	Unit of length - one thousandth of a millimeter (0.001 mm or approximately 1/25,000 inch). This unit is abbreviated as µm.
Microorganisms (Microbes)	Microorganism is the collective name of all living organisms which can only be seen with the aid of a microscope. This group of organisms is very diverse. All bacteria and most protozoa belong to this group, also some algae and fungi.
Molds	Molds are fungi that grow in the form of multicellular filaments (called hyphae). In this vegetative phase, they can produce spores (conidia) which can readily become airborne.
Mycelium (plural: Mycelia)	The vegetative phase of fungi consisting of a network of thread-like hyphae. Mycelia can form a "mat" floating

	at the surface of formulation vessels and other containers.
“Pigs”	Pipeline pigs are devices that are inserted into and travel throughout the length of a pipe driven by a product flow. They were originally developed to remove deposits which could obstruct or retard the flow through a pipeline. Today pigs are used during in the life of a pipe line for many different reasons.
Precipitate	A deposit of solid particles formed after separation of a suspended liquid on the bottom of containers.
Prokaryotic Cells	Bacteria have prokaryotic cells which sets them apart from organisms with the more sophisticated eukaryotic cells. Bacterial cells do not have a nucleus or clear mitochondria. There is normally one single chromosome that floats in the protoplasm.
Sanitization	Destruction of most microorganisms on hard surfaces, in water, raw materials through the use of chemicals, heat and/or irradiation.
Sanitizer (Disinfectant)	A sanitizer is either a chemical or physical agent effective in reducing microbial contamination on product contact surfaces.
Soluble Liquid (SL)	An agricultural formulation in which the salt(s) of the active ingredient(s) is completely dissolved and is applied after dilution in water. Sometimes referred to as soluble concentrate using the same abbreviation - SL.
Spores	Spores of yeasts and molds result from either sexual or asexual reproduction. They are often “designed” for dispersal and survival under unfavorable conditions, often for extended periods of time. Spores form part of the life cycles of many plants, algae, fungi and protozoa. Bacteria form endospores (see endospores).
Supernatant	A liquid standing / floating above a precipitate.
Suspension Concentrate - (SC) (= Flowable concentrate)	A stable suspension of AI(s) with water as the fluid intended for dilution with water before use.

Suspo-emulsion (SE)	A liquid, heterogeneous preparation consisting of a stable dispersion of active ingredient(s) in the form of solid particles and of fine globules in a continuous water phase.
Thickener	A thickener or thickening agent is a co-formulant added to water-based formulations to increase the viscosity of the product and improve the suspension or emulsion of active ingredients. This will aid the stability of the formulation.
Yeasts	Single-cellular microorganisms belonging to the fungi. Reproduction is commonly by means of budding. They use organic compounds (e.g., sugars, organic acids) as energy source.

Appendix – Checklist / Self-Assessment

This self-assessment will help manufacturers and their External Manufacturers to assess the compliance of their manufacturing processes and technical equipment with the key criteria for Prevention and Control of Microbial Contamination, as well as, the competency of their staff. Ideally, the lead auditor should be an outside expert (e.g., the QA manager from a different site of the same parent company, or an independent consultant).

A negative reply to the questions which are directly related to process issues should be followed up by a corresponding action plan to remedy or improve the situation, or an explanation of why an improvement is not necessary.

This checklist can also be used as the section on the Prevention and Control of Microbial Contamination of a client's EM audit check list.

The frequency of the Self-Assessment / EM audit should be determined by each manufacturer and its EM based on their own Microbial Contamination risk assessments and must be adjusted to cover events that impact this risk.

For example, more frequent assessments / audits will be required whenever:

- The sources of raw materials have been changed.
- “Hot spots” have been detected.
- Microbial contamination has been observed in finished products.
- New cleaning and sanitization procedures have been adopted.
- New water-based crop protection products have been introduced.
- The action plan to correct any non-conformity with the Prevention and Control of Microbial Contamination criteria has been completed.

When reliable Prevention and Control of Microbial Contamination can be demonstrated the audit frequency can be reduced.

Contents:

1. Management Responsibility
2. Water, Raw materials. Procurement
3. Product Portfolio
4. Biocides
5. Plant Hygiene, Housekeeping, Cleaning and Sanitization / Disinfection
6. Warehousing
7. Microbiology Tests

1	Management Responsibility	Yes	No	Comments / Details / Proposed Action Plans
1.1	Risk Assessment			
	<p>Did your site perform a micro-biological contamination risk assessment for the manufacturing of water-based crop protection products?</p> <p>If so, does the assessment cover:</p> <ul style="list-style-type: none"> • Personnel • Facility and room design • Process layout • Equipment • Preparation Process Water • Cleaning and sanitization practices <p>Please provide details</p>			
1.2	Standards			
	<p>Does your site have:</p> <ul style="list-style-type: none"> • a Company Standard and standard Guidelines / • Standard Operating Procedures • Company Policy covering microbiological contamination prevention? <p>Please provide details</p>			

1.2	Standards (cont'd)	Yes	No	Comments / Details / Proposed Action Plans
	<p>Are the standards of CropLife International "Prevention and Control of Microbiological Contamination in Crop Protection Products" being implemented?</p> <p>If the company uses other or additional standards, please describe.</p>			
1.3	Responsible persons			
	<p>Do you have appointed a person in your organization for the implementation of "Prevention and Control of Microbial Contamination in water-based Crop Protection Products" methodology?</p> <ul style="list-style-type: none"> • Name(s)? • Time in this role? <p>If so, is this person(s) also responsible for implementation of the prevention of chemical cross-contamination?</p>			
1.4	Training & awareness			
	<p>Do you provide regular training on "Prevention and Control of Microbial Contamination in water-based Crop Protection Products"?</p>			

1.4	Training & awareness (cont'd)	Yes	No	Comments / Details / Proposed Action Plans
	<p>If so, does this training form an integral part of your Quality Awareness training?</p> <ul style="list-style-type: none"> • Is the training documented and archived? • Does importance of personal hygiene get emphasized? In which form? • Is appropriate PPE worn to protect the product from microbial contamination? Please provide details. 			
1.5	Awareness raising activities			
	<ul style="list-style-type: none"> • Describe any additional awareness raising activities. 			

2	Water, Raw materials, Packaging Materials. Procurement	Yes	No	Comments / Details / Proposed Action Plans
2.1	Water Source			
	<p>Please check the water source of the water used on the site in water-based products:</p> <ul style="list-style-type: none"> • Potable water (sourced from the municipality) • Surface water (e.g., River water) • Well water • Rain water <p>Is the water source tested for microorganisms?</p>			
2.2	Preparation process water			
	<p>Is the water used for formulation treated?</p> <p>If so, which treatment methods are used on your site? Please, add a check mark.</p> <ul style="list-style-type: none"> • Filtration • Chemical treatment with disinfectants • Deionization • Reverse osmosis • UV irradiation 			

2.3	Storage of process water	Yes	No	Comments / Details / Proposed Action Plans
	<p>Is the process water stored prior to use?</p> <ul style="list-style-type: none"> • Describe storage conditions, e.g., average temperature, storage time, on a tank farm, in tanks using a closed loop with continuous circulation? • If you store water is it routinely checked for the presence of microorganisms? • If so, please describe your procedure and frequency of measurement. • If you store water does the site have a preventive maintenance cleaning procedure? • If so, what is the frequency, and what are the cleaning & sanitization methods? 			
2.4	Cleaning water			
	<ul style="list-style-type: none"> • Is the water used in manufacturing identical to water used for cleaning? • Is the pH and hardness of process and cleaning water checked and adjusted? • Are rinsates re-used in manufacture and if yes, under which conditions? <p>Please explain</p>			

2.5	Procurement	Yes	No	Comments / Details / Proposed Action Plans
	<ul style="list-style-type: none"> • Do you have specifications in place with your suppliers defining the necessary limits for CFU counts for raw materials (See Chapter 3.5, page 15)? • Are the raw materials tested for CFU count? <p>Please provide details / examples</p>			

3	Product Portfolio	Yes	No	Comments / Details / Proposed Action Plans
3.1	Manufacture water-based crop protection products			
	<p>If you handle any of these aqueous type of products, please list which ones and provide details:</p> <ul style="list-style-type: none"> • Wet cakes of process intermediates? • Formulation concentrates of aqueous formulations? • Soluble liquids (SL)? • Suspo-emulsions (SE)? 			

3.1	Manufacture water-based crop protection products (cont'd)	Yes	No	Comments / Details / Proposed Action Plans
	<ul style="list-style-type: none"> • Suspension concentrates (SC) including in-process slurries for e.g., WG? • Seed treatment formulations (FS)? • Gels for direct application (GD) <p>Are there products manufactured in the pH range of 6 to 8?</p>			

4	Biocides	Yes	No	Comments / Details / Proposed Action Plans
4.1	Biocides			
	Do you know the maximum allowed addition of biocides for all products?			

5	Plant Hygiene, Housekeeping, Cleaning & Sanitization / Disinfection	Yes	No	Comments / Details / Proposed Action Plans
5.1	Cleaning and Sanitization Program			
	<p>Do you have a cleaning and disinfection program in place for:</p> <ul style="list-style-type: none"> • Prevention & control of microbial contamination? • Prevention of Chemical cross-contamination? • Other reasons? <p>Do you use a mechanical cleaning? If so, please explain.</p> <p>Do you use a chemical process? If so, what agents and processes are used?</p> <p>Do you have a sanitization process? What agents and processes are used?</p> <p>Do you clean and sanitize between campaigns?</p> <p>Do you clean between batches?</p> <ul style="list-style-type: none"> • Between each batch? • Or, between “x” batches? 			

5.1	Cleaning and Sanitization Program (cont'd)	Yes	No	Comments / Details / Proposed Action Plans
	<p>• Is the cleaning / disinfection done immediately after the completion of a campaign?</p> <p>Please provide your procedure.</p> <p>Do you have a procedure to make sure the cleaning / sanitization agents are removed?</p> <p>Is there a procedure on how to clean the outside of pipes and fixed equipment, e.g., vessels?"</p>			
5.2	Drying & storage of equipment			
	<p>If the next campaign is not immediately following the previous campaign do you make sure the equipment will be dried completely?</p> <p>Are there parts of the equipment that cannot be dried? Please explain.</p> <p>What do you do to prevent microbial growth in those parts?</p> <p>Do you hang hoses to dry and cap them afterwards?</p> <p>Do you have a procedure for cleaning or replacing filters? Please explain.</p>			

5.2	Drying & storage of equipment (cont'd)	Yes	No	Comments / Details / Proposed Action Plans
	<p>Do you have a procedure for cleaning of sight glasses? Please describe.</p> <p>Do you have a procedure to clean and to store movable equipment, like pumps and beads? Please explain.</p>			
5.3	“Hot Spots”			
	<p>Do you have a map of the production facility including packaging lines on which the areas prone to microbial growth (= “hot spots”) are clearly marked?</p> <p>If yes, please provide details.</p> <p>Do you carry out an inspection of these “hot spots” to check for microbial growth?</p> <ul style="list-style-type: none"> • Before the start of a campaign of water-based products? • Also during the manufacturing process? • Do you have a special cleaning program for the preparation of e.g., thickeners? <p>Please provide your procedures and current results.</p>			

5.4	Critical control points	Yes	No	Comments / Details / Proposed Action Plans
	Have the critical control points within the manufacturing process that have to be monitored for microbial contamination been identified?			

6	Warehousing	Yes	No	Comments / Details / Proposed Action Plans
6.1	Storage requirements / Re-use of pallets			
	<p>Does the warehouse have housekeeping and cleaning procedures to prevent microbiological contamination?</p> <ul style="list-style-type: none"> • Are the raw materials and packaging materials stored in a dry environment? • Are the packaging materials covered and protected against dust? • Are the unused packaging materials (surplus after production run) covered to prevent microbial contamination as soon as possible after the production run? • Does the site recycle / reuse used pallets? <p>If this is the case are they steam treated before use?</p>			

6.1	Storage requirements / Re-use of pallets (cont'd)	Yes	No	Comments / Details / Proposed Action Plans
	<ul style="list-style-type: none"> Does the site recycle any packaging/storage material like IBCs? <p>If so, please explain the cleaning and sanitization procedure.</p>			
6.2	Warehousing policies			
	<p>Do the warehouses have written housekeeping and cleaning procedures to prevent microbial contamination?</p> <p>Are there permanent records of the housekeeping and cleaning activities?</p> <p>Please provide details.</p>			

7	Microbiology Tests	Yes	No	Comments / Details / Proposed Action Plans
7.1	Microbiological test facilities			
	<p>Do you conduct the tests for the presence of microbes:</p> <ul style="list-style-type: none"> • In your own on-site laboratory? • The analyses are outsourced? <p>Please give the name and address of your contract laboratory and preferably of your contact person.</p>			
7.2	Microbiological test methods			
	<p>Which microbiological test methods are used by your company?</p> <p>Please list all</p> <p>Is a CFU-count determined in the formulated product?</p> <p>Does the CFU-count form part of the product specification and is this used for the release decision?</p>			
7.3	Microbiological Screening Program			
	<p>Do you have a routine microbiological screening program in place?</p>			

7.3	Microbiological Screening Program (cont'd)	Yes	No	Comments / Details / Proposed Action Plans
	<ul style="list-style-type: none"> • Is the water supply checked for the presence of microbes? • Are raw materials and intermediates checked for the presence of microbes? • Do you check packaging materials for the presence of microbes? • Do you check materials that need to be reworked for the presence of microbes? • Do you check materials that will be recycled for the presence of microbes? • Before storage? • Before re-introduction in the process? <p>Please give details</p> <ul style="list-style-type: none"> • Do you check the finished products for the presence of microbes; how is it done and how often? <p>Please give details</p>			

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Notes

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Graphic design:



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