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Evaluation of factors influencing disinfection efficacy for aquaculture

Photo: Akvaplan NIVA

Norwegian Institute for Water Research

REPORT

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Summary

Disinfectants falls within the scope of the Biocidal Products Regulation (BPR), however, there are no specific guidance on how to test the efficacy of disinfectants for use in aquaculture. The Norwegian Medicines Agency and the Norwegian Food Safety Authority are the current authorities for approval and follow up on the national regulations of disinfectants for aquaculture in Norway, while the Norwegian Environmental Agency (NEA) will be the authority for the BPR. Thus, this project was funded by NEA for making a guidance document for efficacy testing of disinfectants for use in aquaculture that is in accordance with the BPR and that can be a supplement to the European Chemical Agency's (ECHA) guidance on the Biocidal Products Regulation Volume II Efficacy assessment and evaluation (Part B + C). This report summarizes the aquaculture industry in EU and Norway, and the typical conditions for uses and use patterns for disinfectants in aquaculture. This information was used to make a recommended guidance document on how to perform efficacy testing of disinfectants for use in the EU and EEA in accordance with BPR. The guidance document is a standalone document.

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Evaluation of factors influencing disinfection efficacy for aquaculture

For preparation of the Aquadisinfectants Guidance Document for efficacy testing and evaluations of disinfectants for use in aquaculture in accordance with the Biocide Product Regulation.

Preface

This report summarizes the information collected on aquaculture practices and disinfectant needs and uses in Norwegian and European aquaculture as part of the project "Guidelines for efficacy testing and evaluation of disinfectants for use in aquaculture" as an assignment from The Norwegian Environment Agency. This reports also describes the approach of making the guidance document, which is another deliverance in this project in form of a standalone guidance document named "The Aquadisinfectants Guidance Document - A Guidance Document on the efficacy testing of disinfectants used in Aquaculture in the EU and EEA."

The main contact with the aquaculture industry and disinfectant suppliers or producers has been Kamilla Furseth. Samantha E. Martins has provided a summary of current regulations, assessment of testing strategies and is the main author of the guidance document. August Tobiesen has carried out the assessment of disinfectant sensitivities of different microorganisms. Aina C. Wennberg has collected information about aquaculture in the EU and the different aquaculture pathogens. Ole Kristian Hess-Erga has provided knowledge and advice based on his expertise in aquaculture. The report and guidance document were quality assured by Adam Lillicrap.

Oslo, 16.12.2022

Aina Charlotte Wennberg

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Summary

The Biocidal Products Regulation (BPR, Regulation (EU) 528/2012) is intended to protect human and animal health and the environment at the same time as providing a harmonised set of rules for placing on the market and the use of biocidal products in the EU and EEA. Disinfectants falls within the scope of the BPR, however, there are no specific guidance on how to test the efficacy of disinfectants for use in aquaculture. In Norway, aquaculture is an important industry, and the use of disinfectants for aquaculture is highly regulated. However, the national regulations will be phased out as the BPR is fully implemented. Thus, it is important to ensure that the special conditions and needs for disinfection for the aquaculture industry both in Norway and in EU is implemented in the BPR so that there will be disinfectants that have sufficient efficacy on the market.

The Norwegian Medicines Agency and the Norwegian Food Safety Authority (NFSA) are the current authorities for approval and follow up on the national regulations of disinfectants for aquaculture in Norway, while the Norwegian Environmental Agency (NEA) will be the authority for the BPR. Thus, this project was funded by NEA for making a guidance document for efficacy testing of disinfectants for use in aquaculture that is in accordance with the BPR and that can be a supplement to the European Chemical Agency's (ECHA) guidance on the Biocidal Products Regulation Volume II Efficacy assessment and evaluation (Part B + C).

This report summarizes the aquaculture industry in EU and Norway, and the typical conditions for uses and use patterns for disinfectants in aquaculture. Most of the information on use and disinfection practices was collected from Norwegian aquaculture industry and disinfectant producers. Especially the salmonid industry has been used as examples to identify the different areas of use, target sites and disinfection practices. In addition, information about other aquaculture species and practices from other European countries are also included where information could be obtained. A list of relevant pathogens that are targeted for disinfection in aquaculture was compiled from Norwegian Fish Health reports and from European and international watch lists of aquatic pathogens.

Information on relevant conditions for disinfection practices in the aquaculture industry was used to make a recommended guidance document on how to perform efficacy testing of disinfectants for use in the EU and EEA in accordance with BPR. The guidance document is a standalone document.

Sammendrag

Tittel: Vurdering av faktorer som påvirker desinfeksjonseffekt for akvakultur.
Utarbeidelse av veiledningsdokumentet «Aquadisinfectants» for effektivitetstesting og evalueringer av desinfeksjonsmidler til bruk i akvakultur i samsvar med Biocidforskriften.
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Biocidforskriften (BPR, forordning (EU) 528/2012) har til hensikt å beskytte miljøet og menneskers og dyrs helse samtidig som den gir et harmonisert regelverk for omsetning og bruk av biocidprodukter i EU og EØS. Desinfeksjonsmidler faller innenfor rammen av BPR, men det er ingen spesifikk veiledning om hvordan man skal teste effektiviteten av desinfeksjonsmidler til bruk i akvakultur. I Norge er akvakultur en viktig næring, og bruk av desinfeksjonsmidler til akvakultur er strengt regulert. Det nasjonale regelverket vil imidlertid bli faset ut etter hvert som BPR blir fullt implementert. Det er derfor viktig å sikre at de spesielle forholdene og behovene for desinfeksjon for oppdrettsnæringen både i Norge og i EU blir ivaretatt i BPR, slik at desinfeksjonsmidler som er på markedet har tilstrekkelig effekt.

Statens legemiddelverk og Mattilsynet er nåværende myndigheter for godkjenning og oppfølging av det nasjonale regelverket for desinfeksjonsmidler til akvakultur i Norge, mens Miljødirektoratet vil være myndighet for BPR. Dette prosjektet ble derfor finansiert av Miljødirektoratet for å lage et veiledningsdokument for effektivitetstesting av desinfeksjonsmidler til bruk i akvakultur i samsvar med BPR, og som kan være et supplement til det europeiske kjemikaliedirektoratets (ECHA) veileder (Biocidal Products Regulation Volume II Efficacy assessment and evaluation (Part B + C)).

Denne rapporten oppsummerer oppdrettsnæringen i EU og Norge, og de typiske forholdene for bruk og bruksmønstre for desinfeksjonsmidler innen akvakultur. Det meste av informasjonen om bruks- og desinfiseringspraksis ble samlet inn fra norsk oppdrettsnæring og desinfeksjonsprodusenter. Spesielt laksefisknæringen har blitt brukt i eksempler for å identifisere de ulike bruksområdene og metodene. I tillegg er informasjon om andre akvakulturarter og praksis fra andre europeiske land inkludert der informasjon var tilgjengelig. En liste over relevante patogener for desinfeksjon i akvakultur er laget basert på den norske fiskehelserapporten og fra europeiske og internasjonale overvåkningslister over akvatiske patogener.

Informasjon om relevante forhold for desinfeksjonspraksis i oppdrettsnæringen ble brukt til å lage et veiledningsdokument om hvordan utføre effektivitetstesting av desinfeksjonsmidler i henhold til BPR i EU og EØS. Veiledningsdokumentet er et frittstående dokument.

1 Introduction

The Biocidal Products Regulation (BPR, Regulation (EU) 528/2012) is intended to protect human and animal health and the environment at the same time as providing a harmonised set of rules for placing on the market and the use of biocidal products in the EU and EEA. A biocide is a product that contains active ingredient(s) that are used to protect humans, animals, materials or articles against harmful organisms and pathogens. The regulation concerns both the product and the active ingredient(s) and requires that the product receives authorisation before it can be placed on the market. Authorisation is only granted if the product is shown to be sufficiently effective for the intended use and product type (PT). Disinfectants used within aquaculture falls within the scope of the BPR, however, there are no defined product types and use patterns described for efficacy evaluation of such products in the European Chemical Agency's (ECHA) guidance on the Biocidal Products Regulation Volume II Efficacy assessment and evaluation (Part B + C) [1] (referred to as ECHA Guidance document from now on).

Aquaculture is an important industry in Norway, and best practice disinfection is necessary for the industry both for fish welfare and environmental protection from pathogens. Thus, Norway has national regulations for approval of disinfectants specific for use in aquaculture. These regulations will be phased out with the implementation of the BPR. However, to ensure protection against infectious diseases among farmed fish it is important that disinfectants used in aquaculture are proved to be efficient at the relevant conditions and for the relevant microorganisms. It is also useful for the aquaculture industry in the EU that the disinfectants on the market are sufficiently effective. Infectious diseases are a significant constraint on aquaculture productivity and animal welfare in EU, and improved good husbandry practices and disease prevention are issues to be addressed according to the European Commission (COM/2021/236 final) [2]. Thus, there is a need for a guidance document (GD) to supplement or be included in the ECHA Guidance document on how to perform efficacy testing of disinfectants intended for use in aquaculture.

This report is a summary of the information gathered and the assessments by NIVA in a project for the Norwegian Environmental Agency with the purpose of writing a guidance document on the efficacy testing of disinfectants for use in aquaculture in EU and EEA.

2 Methods

2.1 Overview of aquaculture and aquaculture practises in Europe

In order to get an overview of areas where disinfectants are used in aquaculture, the major aquaculture activities in Europe needed to be mapped. The European commission on Oceans and fisheries was used as source for information on aquaculture activities in Europe together with Eurostat.

2.2 Collecting information on disinfectant approval, use, and use conditions for aquaculture in Norway

Overview of the regulation and testing strategy for efficacy testing for approval of disinfectants in Norway was summarized based on information in regulations, guidance document for application of approval of disinfectants and information from web pages of the relevant authorities in Norway.

Products approved for use in aquaculture in Norway are listed on the web site for the Norwegian Food Safety Authority[3]. The different areas where disinfectants are used in aquaculture were mapped based on information from the disinfectant producers, and this information was supplemented with a description of the conditions during use, based on experience and information from aquaculture industry and disinfectant producers.

2.3 Identifying problematic microorganisms targeted for disinfection in aquaculture

The microorganisms listed in Norwegian regulations on aquaculture, and information on pathogens from the annual Norwegian Fish Health Report, published by The Norwegian Veterinary Institute, were used to summarize important problematic microorganisms in Norwegian aquaculture. The list of pathogens relevant also for the rest of Europe was collected from the list of diseases to be controlled in the EU Animal Health Law[4] and on the watch list of World organization for animal health[5]. However, veterinary reports from aquaculture in other European countries where not consulted. Some of the pathogens in the watch lists are not prevalent in the EU as of yet, but they are considered a risk if imported because of damage they have caused in other parts of the world.

2.4 Comparing problematic microorganisms with standard reference organisms

The standard reference organisms used in CEN standard tests for efficacy and listed in appendix 3 on the ECHA guidance document, were compared to problematic microorganisms in aquaculture in respect to susceptibility and resistance to typical active ingredients in disinfectant products where this information could be obtained from peer reviewed literature.

3 Results

3.1 Aquaculture in EU and Norway

Norway is the leading aquaculture producer in Europe, with higher production volumes than the whole of the EU combined[6], see Figure 1. Within the EU, the countries owning the largest aquaculture production in terms of volume are Spain, France, Italy and Greece[7]. The EU aquaculture production is dominated by shellfish (45%), followed by marine fish (20%) and freshwater fish (20%), with mussels, salmon, seabream, rainbow trout, seabass, oysters, and carp being the most important species[7] (Figure 2). Freshwater fish production is small compared to other finfish in these countries, while salmon, trout and smelts are the dominant production in Norway. According to the Strategic guidelines for a more sustainable and competitive EU aquaculture for the period 2021 to 2030, mollusc farming (mussels, oysters, clams) in the EU is mostly a traditional, family-based, and labour-intensive aquaculture activity[2]. Aquaculture in Norway on the contrary, has become a big and professional industry.

Aquaculture systems include cages, enclosures and pens, ponds, tanks with recirculating systems (RAS) and tanks and raceways (Figure 3). According to the Eurostat's handbook[8], the following definitions apply: **Cages** means installations floating suspended or fixed to the substrate in natural water bodies with natural water interchange through nets, mesh or other porous material. **Enclosures and pens** are separating out a part of a natural or artificial water body by net mesh, fences or other constructions, also allowing natural water interchange. **Ponds** are relatively shallow and usually small bodies of still water that are most frequently artificially formed. Water is usually stagnant, but with periodic water exchange or water flushing, while trout ponds may have a high water replenish rate. **Tanks and Raceways** are constructed units with high water turnover rate and a highly controlled environment, but with flow through of water instead of treatment and recirculation. **RAS** are tanks with a sophisticated system for maintaining water quality by recirculating and treating the water for minimal water footprint. Cages, enclosures and pens can be sea or lake-based, while the rest are defined as land-based aquaculture in this report.

The type, size and organisation of the aquaculture production influence to what extent there are established best practices for infection controls and disinfectant use. There will be more need for disinfectants in closed systems and tanks than in open cages and ponds. The type of production systems in the countries with the largest production is presented in Figure 3[6]. There is apparently no or very little production in RAS in Norway in this figure. However, this is probably because RAS is mainly used for early life stages and juvenile production, while the grow up phase is done in open or semi-closed sea cages.

Based on this overview, we will focus on the production of salmonid fish with respect to disinfection practises as this seems to be the dominant industry with the largest use of disinfectants. In addition, other practices will be included where information are available. We believe that the diverse practices within the salmonid industry that covers both fresh, brackish and seawater will also cover most of the practices of other types of aquaculture.

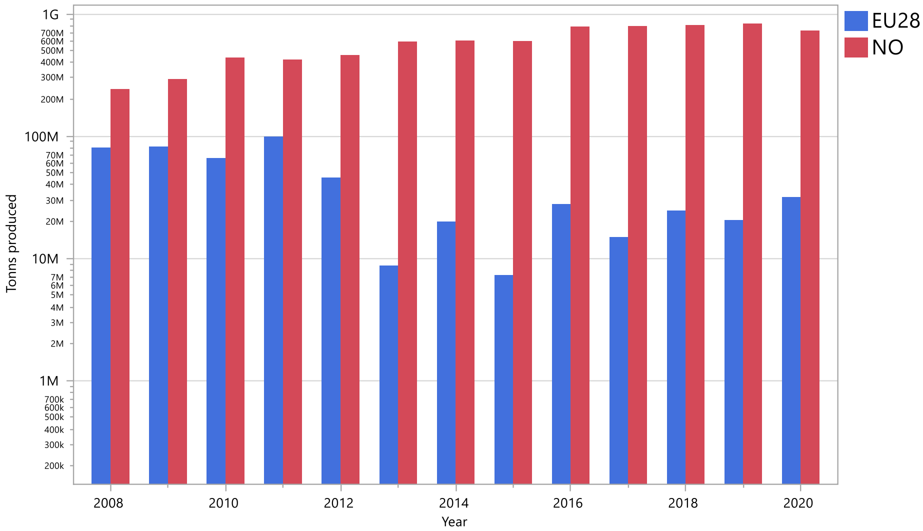

Figure 1: Total volumes of finfish and shellfish produced in aquaculture in 28 EU countries (blue) and Norway (red) from 2008 to 2020. Data collected from Eurostat[6] on 11.10.2022.

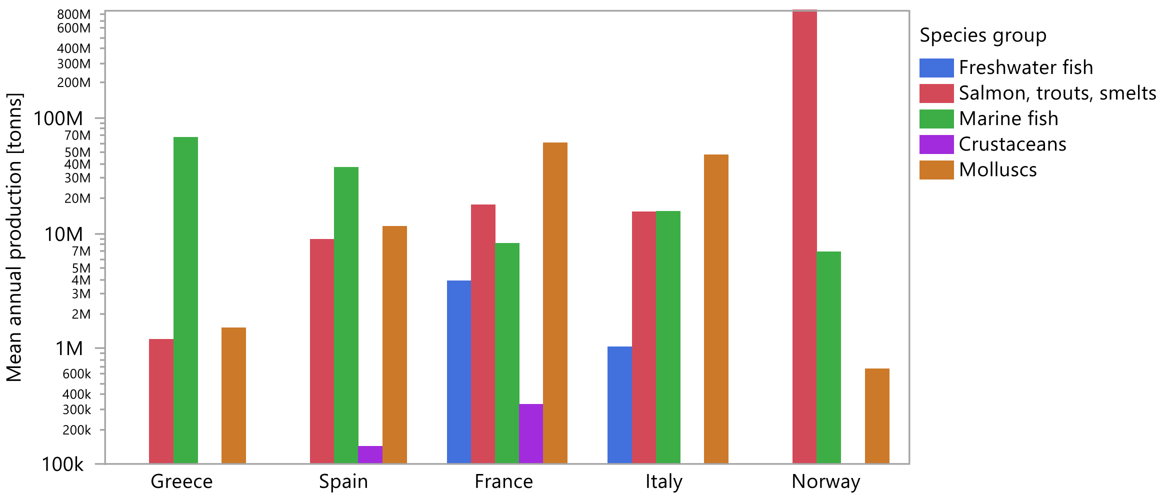

Figure 2: Mean annual production of different groups of aquaculture species groups for the main aquaculture producing countries in the EU and Norway in the period 2015-2020. Data collected from Eurostat[6] on 11.10.2022.

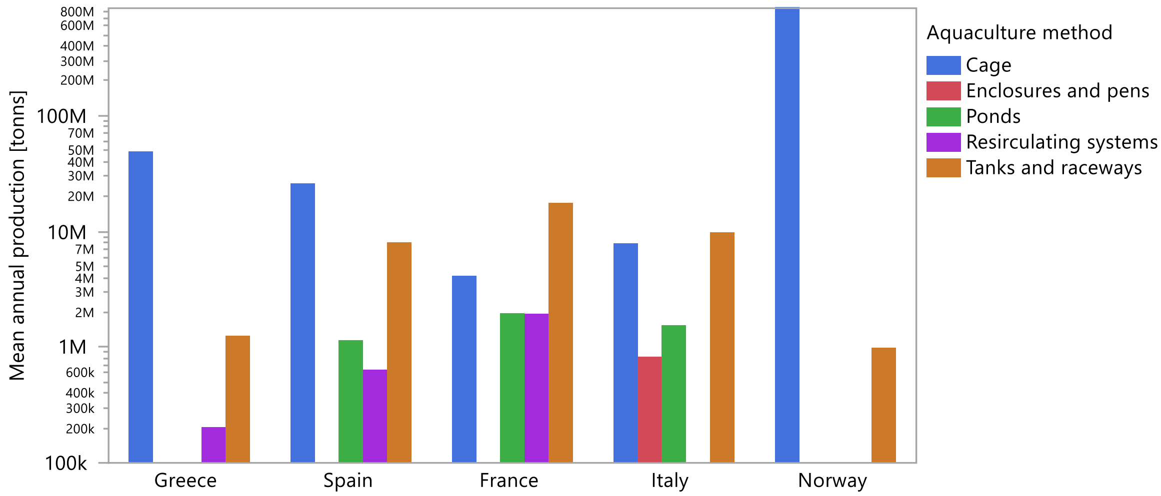

Figure 3: Aquaculture method average production per 2015-2020. Data from Eurostat[6].

3.2 Summary of today's testing strategy for approval of disinfectants in Norway

Disinfectants in Aquaculture are currently approved by the Norwegian Medicines Agency with supervision by the Norwegian Food Safety Authority (NFSA). The Norwegian law regulates the use of disinfectants for aquaculture through the Regulation on disinfection of intake water and waste water from aquaculture-related activities (FOR-1997-02-20-192) and the Regulation on the approval and use of disinfectants in aquaculture facilities and transport units (FOR-2008-06-17-821).

Requirements for disinfection in aquaculture include disinfection of objects, used equipment, transport units, facilities, water and fertilized salmon roe. The NFSA supervises that disinfection is carried out in accordance with regulations and that approved disinfectants and disinfection methods are used[3].

If the disinfection applies to one or more named diseases, the disinfectant must be approved for use against the relevant group of infectious agents (bacteria, viruses or fungi). If there is no approved disinfectant that is suitable in connection with eliminating the pathogen from water and/or facilities during or after an outbreak, the NFSA can give permission for other preparations or active ingredients to be used if these have been tested against the specific infectious agent.

Disinfection of intake and effluent waters is required in facilities that hatch and produce salmon and other freshwater fish, slaughterhouses and manufacturing facilities, as well as land-based aquaculture facilities that have permission to run infection tests with aquatic organisms. The transport water for well boat transport of fish to an aquaculture facility also should undergo disinfection before it is taken into the well (intake water) and discharged from the well boat (effluent water)[10].

In hatcheries, freshly fertilized salmon roe must be disinfected before they are incubated. Roe of species other than salmon must also be disinfected if a suitable disinfection method is available. Although requirements for roe disinfection methods are not set up in the Regulations, the NFSA provides disinfection recommendations[11].
The current approved methods for disinfection of different target sites are summarised in Table 1. Specific conditions can be found in the corresponding regulatory/guidance documents. The methods include both the use of disinfectants and physical treatments such as UV irradiation and filtration.

Table 1. Disinfection methods in Norwegian Aquaculture

Target site	Approved methods	Reference for specific conditions
Intake water	UV irradiation Ozonation Filtration	12
Effluent water	Formic acid Sodium hydroxide Chemical precipitation + UV irradiation Mechanical separation + chlorination Chemical precipitation + chlorination Heat treatment Filtration	12
Salmonid roe	Iodophor	11
Marine species roe	Glutaraldehyde Ozonation	11

3.3 Uses and use patterns for disinfectants in aquaculture in Europe

There are different needs and regulations for disinfection in the aquaculture industry, related to the farming technology, equipment and the type of water. The target areas consist of different farming technology, such as flow-through farms and recirculating aquaculture systems (RAS), but also slaughterhouse, processing plants, research facilities and transport. All of these are collected under the category "land-based" in the following text. If there are regulations covering parts of the land-based operations, this will be specified. Sea based farming also requires disinfection, mostly equipment such as nets, boats, handling equipment and personal equipment. Disinfection of such items is mostly performed under a wider range of environmental conditions (weather, temperature etc.) than those under the category "land-based". Well boat operations are regulated separately in Norway[13] and in Scotland[14].

Ultraviolet radiation (UV) and ozone are frequently used to disinfect water, especially intake and effluent water in aquaculture operations. Discharges from processing plants/slaughterhouses, farms with permission to perform infection tests and dead fish/leftovers from processing, often require other disinfection methods, such as high/low pH, chlorination and elevated temperatures, to reach the required disinfection efficiency. Equipment and surfaces are mostly treated with disinfectants administered by foam, spray or immersion.

Biological matter, such as fish roe, is also disinfected. Disinfection of roe is required in some European countries, such as Norway and Scotland, following recommendations set by the national authorities. For salmonid fish the recommendations are clear, other fish species are somewhat less regulated. The roe of seabass and seabream are also disinfected to some extent, but whether the activity is regulated by law in the producing countries is unclear. The practise of disinfection of roe is in the border between disinfection practices and veterinary medicine. Whether the product is considered a biocide or a veterinary medicine depends amongst others on the specific use and product claims.

An overview of target sites and conditions where disinfectants are used are provided in Table 2.

Table 2. Typical conditions for disinfecting.Based on Norwegian regulations and typical conditions.

Target sites	Use pattern (claim)	pH	Temp.(°C)	Salinity	Soiling	Method of disinfecting	Material to be disinfected	Relevant use area
Water	Intake water, freshwater	6-7	3-20	0-3	-	UV, ozone	Water	Land based, well boats
Water	Intake water, brackish/estuarine	7-8	3-20	10-26	-	UV, ozone	Water	Land based, well boats
Water	Intake water, seawater	7-8	3-20	30-35	-	UV, ozone	Water	Land based, well boats
Water	Recirculating water, freshwater	6-7	3-20	0	-	UV, ozone	Water	Land based
Water	Recirculating water, brackish	7-8	3-20	10-26	-	UV, ozone	Water	Land based
Water	Recirculating water, seawater	7-8	3-20	30-35	-	UV, ozone	Water	Land based
Water	Effluent water, high organic load freshwater	6-8	3-20	0-35	High	pH-treatment, UV, chlorination	Water	Land based (processing plant/slaughterhouse)
Water	Effluent water	6-8	3-20	0-35	Some	Temperature treatment, chlorination, UV	Water	Land based (farms with permission to perform infection tests)
Water	Effluent water	6-8	3-20	0-35	some	UV	Water	Well boat (transport and treatment water)
Surface	Large equipment (i.e., boats, pumps)	6-8	-	0-35	some	Foaming, spray, immersion	Metal, plastic	Land based, sea based, well boats
Surface	Handling equipment (i.e., nets, weights, buckets)	6-8	-	0-35	some	Foaming, spray, immersion	Metal, plastic, textile (the nets)	Land based, sea based, well boats
Surface, textile	Personal equipment (i.e., Boots, clothes)	6-8	-	0-35	some	Boot bath, machine wash	Rubber, textile	Land based, sea based, well boats
Surface	Pipelines	6-8	1-20	0-35	some	In water	Pipes	Land based, well boats
Roe	Roe, freshwater fish	6-7	1-8	0	none	Immersion	Roe	Land based (hatchery)
Roe	Roe, marine fish	7-8	1-8	31-34	none	Immersion	Roe	Land based (hatchery)
Organic matter	Ensiling	<4	-	0-35	some	Immersion	Mortalities and viscera	Land based, sea based

(-) indicate that information was not found

3.4 Disinfectants used in Norwegian aquaculture

Aquaculture in Norway is highly regulated, especially concerning spreading of disease and infections. Disinfection is a part of these regulations and the demand for disinfecting is well described by the regulations, either by law or regulations set by authorities. The NFSA have made several guides how to achieve correct disinfection. These are tools helping the farmers with the disinfecting procedures, as well as the importance of increasing the understanding and knowledge about the regulations. Included in these guides are also specific guides for well boats (guides can be found at Mattilsynet.no). Which products that are approved for use in aquaculture is also regulated by the NFSA, see link for updated list of products[3].

Most of the disinfection regulations concern fin fish, and more specific salmonid fish. As previously indicated, salmonid fish is the biggest aquaculture industry in Norway, but there are other species being reared, such as Atlantic halibut, cod, lumpfish and ballan wrasse. Rearing of species that are not fish is not as highly regulated. Examples of such lesser species are European lobster, blue mussels and different types of seaweed. To our knowledge, such species are more commonly produced in flow-through farms or in farms with a combination with flow-through or RAS technology, or directly in the sea.

When talking to farmers about their disinfection procedures they all said that they follow the instructions from the disinfectant producers. This is also what Lazado and Good (2020) [15] found when they surveyed the disinfection strategies in on-land RAS facilities in Norway. They also found that the choice of products is decided by efficacy and the user safety.

Not only equipment and structures need to be disinfected – also fish roe are disinfected. Either before incubation, before transport or both, but there are exceptions. Roe from salmonid fish are incubated in freshwater and disinfected using the approved chemical iodophor[11], under conditions that maintain the roe´s welfare. Iodophor is used in other countries for salmonid fish, such as in Scotland. The Norwegian regulations states that roe from other species than salmonid fish should be disinfected if there is a suitable disinfection methods available (akvakulturdriftsforskriften FOR-2008-06-17-822 §11).

For non-salmonid marine fish roe there is no such specific regulation in Norway, and the disinfection practice between the different species that are reared is mostly imposed by the aquaculture industry. Glutaraldehyde and ozone were the most com[8]mon disinfection methods, but since glutaraldehyde is not safe for humans it is being replaced with other substances, or the roe are not subjected to disinfection at all. Cod fish roe, for example, are not disinfected under the national breeding programme in Norway run by Nofima (pers. comm. Øyvind Johannes Hansen, 14.10.2022). The reasoning is that marine roe tends to have shorter incubation period than salmonid roe and many are not held in incubators in the same way. Alternatively, iodophor is used as a disinfectant on marine roe of lumpfish, cod and spotted wolffish (pers. comm. Atle Foss, 07.10.2022), and there are a few different practices regarding Atlantic halibut. Live feed for marine fish may also need disinfecting, which is performed with the same product as used for disinfecting roe (pers. comm. Kjetil Solheim, 10.10.20.22).

One of the challenges related to disinfection products in Norway is the range of temperatures. Products used in water, to disinfect pipelines or tanks, will be at the production temperature for the species, most commonly ranging from 3-15^{o}C depending on the species and time of year, but also up to 20^{o}C as for European lobster (pers. com. Asbjørn Drengstig, 14.10.2022). Products used for surface disinfection, that are applied by spraying, can be exposed to extreme temperatures when used outside.

Fish farms in Norway are located from the extremes of Kirkenes in the north to Lindesnes in the south, and the outside air temperatures could range from -30°C in winter to 25-30°C in summer depending on the location.

3.5 Problematic microorganisms targeted for disinfection in aquaculture

Disease causing microorganism in aquaculture can be either naturally occurring environmental organisms (opportunistic pathogens) or strictly pathogens. The pathogens of most concern for European fisheries and aquaculture are put on a watch list, with the intention of preventing import to Europe, prevent spread or to eradicate. In case of an outbreak of a pathogen, it is important to disinfect all equipment and water in contact with infected fish/animals to prevent spread. Opportunistic pathogens are often present in aquaculture without causing disease, unless there are favourable conditions for the organism, and/or unfavourable conditions for the host animal, i.e., the host animal is stressed or unhealthy. Disinfection of surfaces and equipment is used to prevent prevalence of high levels of opportunistic pathogens in contact with the aquaculture organisms.

As a precautionary best practice, it is normal to disinfect equipment and personal protective clothes that might be in contact with multiple tanks or cultures of organisms in case there are pathogens or opportunistic pathogens at a site. Thus, disinfectants should be effective against disease causing microorganisms from commonly occurring pathogens and pathogens on the watch lists.

3.5.1 Bacteria

Bacterial infection in aquaculture organisms can be caused by environmental bacteria (opportunistic pathogens) such as mycobacteria or by pathogenic bacteria that rely on a host for growth. Bacteria can be found in the water or in biofilm on surfaces in aquaculture. A list of the most important problematic bacteria for aquaculture in Europe is listed in Table 3. The list is dominated by bacteria infecting fish, and especially salmonids since the Norwegian aquaculture has been the dominating source of information. All the pathogenic bacteria are gram negative, while the environmental opportunistic bacteria family Mycobacterium are gram positive. *Yersinia ruckeri* and *Aeromonas salmonicida salmonicida* are mentioned in Norwegian regulation for intake and discharge water for aquaculture[16]. These bacteria are also included as test organisms in the Norwegian disinfectant approval as typical fish pathogens.

Table 3: Disease causing bacteria found in Norwegian aquaculture or on watch lists in Norway and EU.

Species	Description	Consequence	Type of aquaculture activity	Source of information
Aeromonas salmonicida salmonicida	Pathogen, Gram-negative	Furunculosis	Salmonid fish, all life stages and salinities.	16,17
Flavobacterium psychrophilum	Pathogen, Gram-negative	Bacterial cold-water disease (BCWD) systemic disease	Norwegian endemic, Fish in fresh and brackish water worldwide.	17
Renibacterium salmoninarum	Pathogen, Gram-positive	Bacterial kidney disease (BKD)	Young salmonid fish	17
Pasteurella sp. , *Pasteurella skyensis*	Pathogen, Gram-negative	Pasteurellosis	Salmon and lumpfish	17
Moritella viscosa *Tenacibaculum* sp.	Pathogen, Gram-negative	Winter ulcer	Atlantic salmon, cod, halibut.	17
Yersinia ruckeri	Pathogen, Gram-negative	Yersiniosis, enteric redmouth disease,	Fresh and marine fish, salmonids	16, 17
Vibrio salmonicida	Pathogen, Gram-negative	Cold water vibriosis	Atlantic salmon, cod,	17
Mycobacterium chelonae *M. salmoniphilum* *M. fortuitum* *M. marinum* *M. shottsii,* *M. pseudoshottsii,* *M. salmonipilum*	Environmental Gram-Positive	Mycobacteriosis	Fresh and marine, fish, molluscs, crustaceans	17

3.5.2 Viruses

Viruses need a host organism to proliferate. Table 4 lists the most important problematic viruses for aquaculture in Europe. The list is dominated by viruses that infect salmonid fish but also include viruses that infect other fish and crustaceans. There is a wide range of virus types that infect aquatic species, and the list includes both positive (+) and negative (-) single (SS) and double (ds) stranded RNA and DNA virus. ISAV (-ssRNA) and IPNV (ds RNA) are mentioned in Norwegian regulation for intake and discharge water for aquaculture. More importantly, there are both enveloped and non-enveloped viruses, the latter being more resistant to disinfection.

In the EU more than 45% of aquaculture is shellfish. Related to that, one of the key challenges identified for mollusc farming is the human norovirus[2], as this will cause food poisoning from raw consumption by humans. Thus, it is not only pathogenic organisms to aquaculture animals that are problematic for aquaculture. However, since this virus contamination mostly occurs during production in natural waters, human norovirus is not likely to be targeted by disinfection practices strictly related to aquaculture activities.

Table 4. Disease causing viruses found in Norwegian aquaculture or on watch lists in Norway and EU. The types describe genome structure as positive (+) or negative (-) single stranded (SS) or double stranded (ds) RNA or DNA.

Species	Type	Consequence	Type of aquaculture activity	Source
Epizootic haematopoietic necrosis virus (EHNV)	-SS RNA	Epizootic haematopoietic necrosis	Salmonid fish, carps, wild freshwater fish	4 5
Infectious salmon anaemia virus (ISAV)	-SS RNA envelope	Infectious salmon anaemia (ISA)	Salmonid fish, fresh and seawater	17 16 4
Infectious hematopoietic necrosis virus (IHNV) and Viral haemorrhagic septicaemia virus (VHSV) *Novirhabdovirus* spp. (family Rhabdoviridae)	-SS RNA envelope	Infectious hematopoietic necrosis (IHN) viral haemorrhages septicaemia (VHS)	Salmonid fish, marine and freshwater fish, Europe, not Norway	17 5
Salmonid alphavirus (SAV)	+SS RNA envelope	Pancreas disease (PD)	Salmonid fish	17 5
Nodavirus	+SS RNA non-enveloped	Viral encephalopathy and retinopathy in fish, white tail disease in crustaceans	Marine fish and crustaceans	17 5
Infectious pancreases necrosis virus (IPNV) *Aquabirnaviridae* spp. (family Birnaviridae)	ds RNA non-enveloped	Infectious pancreases necrosis, IPN	juvenile salmonids	17 16
Piscine myocarditis virus (PMCV)	ds RNA envelop	Cardiomyopathy syndrome (CMS)	Atlantic salmon	17
Piscine orthoreovirus (PRV)	ds RNA non-enveloped	Heart and Skeletal Muscle Inflammation (HSMI)	Atlantic salmon	17
Salmon Gill Pox Virus – SGPV	ds DNA	Salmon Gill Pox	Atlantic salmon	17
Taura syndrome virus (TSV)	+SS RNA non-enveloped	Taura syndrome in crustaceans	Marine shrimps: *Penaeus vannamei, P. stylirostris, P. cannamei*	4
Yellow head virus (YHV)	+SS RNA envelope	Yellowhead disease in crustaceans	Marine shrimps and prawns	4
Koi herpesvirus disease (KHVD)	ds DNA envelope	Koi herpes virus disease	Freshwater carp, temperatures above 16°C	4,5
white spot syndrome virus (WSSV)	ds DNA envelope	White spot disease in crustaceans	Mostly prawns (prawns, lobsters and crabs from marine, brackish or freshwater)	4

3.5.3 Fungi

Fungi and moulds are often present in natural waters and often form spores that are more resistant to disinfection. Some species, such as in the genus *Saprolegnia*, can infect fish or fish eggs. Infection of fish is often a result of fish being stressed or have wounds and/or as secondary infections. Disinfection, targeting fungi, is normally for protection of roe or after an outbreak of fungal infections. *Aphanomyces* is a genus of mould of emerging concern. They are not prevalent in Europe yet, but have been found in ornamental fish[18]. A list of the most important problematic fungi for aquaculture in Europe is listed in Table 3.

Table 5. Disease causing moulds and fungi found in Norwegian aquaculture or on watch lists in Norway and EU.

Species	Consequence	Type of aquaculture activity	Source of information
Saprolegnia spp.	Mycosis	Salmonid fish, freshwater fish, roe of salmon.	17
Aphanomyces invadans or *A. piscicida*	Epizootic ulcerative syndrome (EUS)	Wild and farmed freshwater and estuarine fish	4,5
Other examples include species from the genus *Fusarium, Penicillium, Exophiala, Phialophora, Ochroconis, Paecilomyces, Ichthyophonus* and *Lecanicillium*	Environmental fungi that can cause different diseases	Examples are salmonids, other fish species, shrimps	17

3.5.4 Parasites

Parasites are a wide range of organisms, spanning from small animals such as crustaceans (e.g. salmon louse) and cnidarians (Myxozoa) with multi life stages, to single cell protists. A list of the most important problematic bacteria for aquaculture in Europe is listed in Table 6.

Sea and salmon lice at adult life stages are normally removed mechanically by filtration, or through the use of chemotherapeutics regulated as veterinary medicinal products and as such are not subjected to disinfection as control mechanisms.

The flat worm *Gyrodactylus salaris* is endemic in some waters in Norway and Europe. Hobby fishermen are instructed to disinfect equipment, boots and boats used in infected water before using in non-infected water. In this sense, disinfection of this parasite is not necessarily related to aquaculture activities.

Table 6. Disease causing parasites found in Norwegian aquaculture or on watch lists in Norway and EU.

Species	Type	Consequence	Type of aquaculture activity	Source
Salmon lice *Lepeophtheirus salmonis*	Crustaceans	Parasitic infections in fish	Marine fish	[17]
Gyrodactylus salaris	Flat worms	Parasitic infections in fish	Freshwater salmonids	[5, 17, 16]
Salmoxcellia vastator	Protist	Parasitic infections in fish, spoiling effect on fillet	Marine fish	[17]
Sea lice *Caligus elongatus*	Crustaceans	Parasitic infections in fish	Marine fish	[17]
Parvicapsula pseudobranchicola	Myxozoa	parvicapsulosis	Atlantic salmon	[17]
Paramoeba perurans	Protist	Amoebic gill disease (AGD)	Marine fish	[17]
Bonamia exitiosa, B. ostreae, Microcytos mackini, Perkinsus marinus, Marteilia refringens	Protist	Parasitic infections in oysters or other shellfish	Oysters	[4, 5]

3.6 Comparison of reference test organisms and aquaculture pathogenic microorganism

To find out whether the reference test organisms (Table 7) recommended to be used in CEN standard tests (referred to as "standard test organisms" in this document) adequately represent the fish pathogens that need to be treated in aquaculture industry, a literature search (Google scholar) comparing studies in which the efficacy of active ingredients (AI) in disinfectants was tested using standard test organisms (described in EN1656, EN14675 and EN14349, see

Table 7) with studies in which fish pathogens were exposed to the same AI (Table 8 and Table 9).

Published studies comparing fish pathogens with standard test organisms used in efficacy studies are lacking and a very few CEN standard test studies are published in open literature, making comparisons difficult. Direct comparison was only possible for a few AI; namely Peracetic acid (PAA) + Hydrogen peroxide (H_2O_2) and peroxymonosulphate regarding viruses (Table 8) and peroxymonosulphate regarding bacteria (Table 9). In both cases, the standard test organisms required equal or higher dosage to achieve a lg reduction >4. It is important to mention that although comparisons were made, the test conditions were not the same among test species. Due to these challenges, it was not possible to conclude whether the use of standard test organisms is (or is not) adequate to evaluate efficacy of disinfectants intended to be used in aquaculture. Therefore, if there is a real concern that pathogens are less sensitive towards disinfectants in use in aquaculture, then individual producers of disinfectant products could be approached and asked to present their CEN standard test results.

Table 7. Standard reference test species used in standard test methods for disinfection efficacy testing.

Organism group	Test species	Description	Standards
Bacteria	*Staphylococcus aureus* ATCC 6538	Gram positive	EN1656, EN14349, EN16437
	Pseudomonas aeruginosa ATCC 15442	Gram negative	EN1656, EN14349, EN16437
	Enterococcus hirae ATCC 10541	Gram negative	EN1656, EN14349, EN16437
Mycobacteria	*Mycobacterium terrae* ATCC 15755	Gram positive	
	Mycobacterium avium ATCC 15769	Gram positive	EN14204
Yeasts	*Candida albicans* ATCC 10231		EN1657, EN16438
Fungal spores	*Aspergillus brasiliensis* ATCC 16404		EN1657, EN16438
Viruses	Polio virus type 1, LSc-2ab (Picornavirus)	+ssRNA, non-enveloped icosahedral capsid	
	Bovine enterovirus ATCC VR-248	+ssRNA, non-enveloped icosahedral capsid	EN14675
	Porcine Parvovirus NADL2	ssDNA	EN17122
	Adenovirus, ATCC VR-5	dsDNA, non-enveloped icosahedral nucleocapsid	
	Murine norovirus, strain S99 Berlin	+ssRNA, non-enveloped icosahedral capsid	
	Vaccinia virus strain Elstree ATCC VR-1549		EN14675, modified

Table 8: Comparison of sensitivity/tolerance of viruses towards disinfectants used in aquaculture. Test condition: Temperature, Water quality: HW=Hard water as defined in EN standard, FW=Fresh water, SW=Sea water, + albumin= added albumin as defined in CEN standard. Dose is given as amount of active substance and exposure period. Lg reduction indicate lg 10 reduction in viable test organisms relative to a control. PAA=peracetic acid, H_2O_2=Hydrogen peroxide

Species	Active ingredient	Test condition (temp, hardness/salinity, soiling)	Dose: concentration x contact time	Lg reduction	Ref.
SAV	Peroxy-mono-sulphate	4°C, HW, +albumin (EN14675)	2g/l x 5 min	>4	19
		10°C, HW, +albumin (EN14675)	2g/l x 5 min	>4	
		4°C, HW, +albumin (EN14675)	2g/l x 5 min	>4	
		10°C, SW, +albumin (EN14675)	3g/l x 5 min	>4	
		4°C, SW, +albumin (EN14675)	3g/l x 5 min	>4	
		10°C, HW, +albumin (EN14675)	3g/l x 5 min	>4	
IPNV		4 °C, HW, +albumin (EN1656)	0.5 g/l x 30min	>4	20
Enterovirus Polio I Sabin		23 °C,FW (AFNOR)	5g/l x 5 min	>7	21
SAV	Iodine	4°C, HW, +albumin (EN14675)	75 mg/l x 15min	>4	19
		10°C, HW, +albumin (EN14675)	300 mg/l x 30min	>4	
		4°C, HW, +albumin (EN14675)	300 mg/l x 30min	<4	
IPNV		4 °C, HW, +albumin (EN1656)	9 mg/l x 30min	>4	20
ISAV		4 °C, HW, +albumin (DEFRA)	2.4 g/l x 5min	>4	22
IPNV	PAA+H_2O_2	4 °C, HW, +albumin (EN1656)	0.8 g/l x 30min	>4	20
ISAV		4 °C, HW, +albumin (DEFRA)	50 mg/l x 5 min	>4	22
Bovine enterovirus		10 °C, HW, +Albumin (EN14675)	60 mg/l x 30min	>4	23
Hepetatis A virus		10 °C, HW, +Albumin (EN14675)	4.2 g/l x 30min	>4	23
Bovine enterovirus	Chlorine	10 °C, HW, +Albumin pH 9,6 (EN14675)	0.6 g/l x 30min	>4	23
Hepetatis A virus		10 °C, HW, +Albumin pH 9,6 (EN14675)	0.6 g/l x 30min	>4	23
Bovine enterovirus	potassium mono-per-sulphate	10 °C, HW, +Albumin (EN14675)	0.47 g/l x 30min	>4	23
Hepatitis A virus		10 °C, HW, +Albumin (EN14675)	1.5 g/l x 30min	>4	23

Table 9. Comparison of sensitivity/tolerance of bacteria towards disinfectants used in aquaculture. Test condition: Temperature °C, Water quality: HW=Hard water as defined in EN standard, FW=Fresh water, SW=Sea salt water, + albumin= added albumin as defined in EN standard. Dose is given as amount of active substance and exposure period. Lg reduction indicate lg 10 reduction in viable test organisms relative to a control.

Species	Active ingredient	Test condition (temp, salinity/hardness, soiling)	Dose: concentration x contact time	Lg reduction	Ref.
A. salmonicida	Peroxy-mono-sulphate	5-23 °C, SW	6g/l x 1 min	>4	24
		4 °C, HW, +albumin (EN1656)	0.5 g/l x 30min	>4	20
Y. ruckeri		4 °C, HW, +albumin (EN1656)	0.5 g/l x 30min	>4	20
Staphyllococcus aureus		23 °C,FW (AFNOR)	5g/l x 5 min	>5	21
Pseudomonas aeruginosa		23 °C,FW (AFNOR)	5g/l x 5 min	>5	
E. coli		23 °C,FW (AFNOR)	5g/l x 5 min	>5	
Enterococcus hirae		23 °C,FW (AFNOR)	5g/l x 5 min	>5	
Baccilus cereus		23 °C,FW (AFNOR)	5g/l x 5 min	<5	
Mycobacterium smegmatis		23 °C,FW (AFNOR)	5g/l x 5 min	>5	
A. salmonicida	Glutar-aldehyde	5-23 °C, SW,	20 g/l x1 min	>4	24
A. salmonicida	$PAA+H_2O_2$	22 °C, SW,	2 mg/l x 5 min	>4	25
Y. ruckeri		22 °C, SW,	2 mg/l x 5 min	>4	
A salmonicida		4 °C, HW, +albumin (EN1656)	0.05 g/l x 30min	>4	20
Y. ruckeri		4 °C, HW, +albumin (EN1656)	0.1 g/l x 30min	>4	
A salmonicida	Iodine	4 °C, HW, +albumin (EN1656)	80 mg/l x 30min	>4	20
Y. ruckeri	Iodine	4 °C, HW, +albumin (EN1656)	80 mg/l x 30min	>4	
A. salmonicida	Ozone	22 °C, SW,	0.1 mg/l x10 min	3	26
A. salmonicida	Chlorine	22 °C, SW,	0.1 mg/l x 10min	3	26

4 Discussion

4.1 Typical use conditions of disinfectants to find worst case testing conditions for water quality / soiling

Experience gathered during testing of various ballast water treatment systems at NIVA indicate that freshwater or seawater does not change the efficacy of the treatment with regard to use of chlorine, ozone or UV. However, increasing the amount of organic carbon reduces the efficacy of all 3 methods and therefore requires increased levels of disinfectants to compensate. Efficacy reduction is caused because the oxidative power of chlorine and ozone is spent on oxidizing organic compounds.

With regard to organic disinfectants the case is more open. In Table 8 it was observed that 2g/l peroxymonosulphate was enough to give a >4 lg reduction in freshwater for salmonid alpha virus while 3 g/l was necessary for seawater. As it is unlikely that whether it is freshwater or seawater is of no consequence when testing efficacy of organic disinfectants, documentation should be provided for both use patterns if the product is to be approved for use in both environments.

4.2 Suggested test organisms for efficacy testing

There are limited data available for comparing aquaculture pathogens to standard test organisms regarding their sensitivity to active ingredients in disinfectants. The literature review on lg reductions (Table 8 and Table 9) showed differences so large in test conditions related to temperature, soiling and salinity that it was not possible to conclude which species is more sensitive or tolerant to disinfection, if there is one. With that in mind, two main factors were considered for the recommendation of test species:

1. Both for the purpose of comparing results and for acceptance of data, reference organisms described in CEN guideline tests should be used. Newly isolated cultures can be of strains with a-typical traits, and cultures kept in the laboratory for several generations might change behaviour and traits. Thus, recognized culture collections that characterize their cultures (Type cultures) such as the American Type Culture Collection (ATCC) ensure that the species and strain have the expected behaviour typical of that strain.

2. In the case that a non-standard test organism needs to be used, a clear justification should be provided. One example is given: If the aquaculture pathogen is more tolerant than the standard type cultures at typical use conditions, then the disinfection will fail if the dose and application conditions for the disinfection product are determined based on a reference organism that is more sensitive than the actual pathogen. Thus, if either the testing conditions are more unfavourable for the reference organism, or there are typical pathogenic organisms that are more tolerant than the reference organism, the target pathogen or an organism with the same characteristic as the pathogen should be used as test organism.

4.2.1 Bactericidal testing

The reason for including *A. salmonicida* and *Y. ruckeri* for disinfection approval in Norwegian regulation is as far as we know not based on an assumption that these bacteria are more tolerant than standard test organisms, merely that they are aquaculture relevant species (personal communication, Rolf Hovik, 15.11.2022). Most fish pathogens are gram negative bacteria, which are normally more susceptible to disinfection than gram-positive bacteria. Thus, using standard bacteria species, which include both a gram-positive and gram-negative bacterium, or even using only gram-positive bacteria, should be sufficient for efficacy testing and a bactericidal claim for the disinfectant. For a specific claim against mycobacteria, a mycobacterial strain should be tested.

4.2.2 Virucidal testing

The standard EN 14675 test for virucidal activity in the veterinary area uses the Bovine Enterovirus Type 1 (ECBO), a non-enveloped virus, as the test species. Alternatively, a virucidal activity against enveloped viruses can be claimed when *Vaccinia virus* is tested in a (modified) EN 14675 test.

According to the ECHA guidance document, when the test against ECBO passes the criteria, virucidal activity can be claimed, while when only virucidal activity against enveloped viruses is demonstrated the claim cannot be “virucidal activity”.

The viruses relevant for aquaculture include both enveloped and non-enveloped viruses, so this approach should be followed also for aquaculture. The two viruses included for disinfection approval in Norwegian regulation included the enveloped ISAV and the non-enveloped IPNV. The reasons for including IPNV in the efficacy testing and requirements in Norway is that this virus is more tolerant than other fish pathogens in salmon aquaculture (personal communication, Rolf Hovik, 15.11.2022). Thus, if the disinfection product claims include salmonid aquaculture, this virus should be included as a test organism for the virucidal claim.

4.2.3 Fungicidal testing

Not enough data was found to suggest that there are specific fungal species related to aquaculture to warrant testing of the specific species. The genus *Saprolegnia* has been a problem especially for freshwater fish in Norway[27]. However, there are several species in this genus, so a specific claim for the genus must be followed up with documentation that the selected species for testing is both relevant and representative. There are multiple environmental fungi that can cause infections or secondary infections in aquaculture; thus, a general fungicidal claim should be supported with testing with the reference organism stated in the standard test guidelines.

4.2.4 Yeasticidal testing

No information of specific yeast related to aquaculture has been found during this study, thus, yeasticidal claim should be supported with testing with the reference organism stated in the standard test guidelines.

4.2.5 Parasite testing

It should not be possible to make a general claim for parasites, since this is not a defined organism group, but a wide variety of organism spanning from single cellular protists to multi life stages crustaceans. Thus, a claim for parasites must be directed to the specific parasite, and that parasite should then be the test species in the efficacy test.

It is important to note that products against parasites may be considered veterinary medicinal products (VMP). If the product is classified as a VMP or if it is under investigation within the scope of the Veterinary Medicinal Products Directive (2001/82/EC as amended by 2004/28/EC), it is excluded from the BPR for the respective use, therefore efficacy tests are dismissed.

4.3 Standard tests

The CEN standards are designed to test efficacy of a freshly prepared treatment solution, however several fields of application include reuse of treatment solution, typically this will be relevant for categories involving immersion, for example, disinfection of instruments, equipment and nets. In addition, disinfection of personal equipment such as boot baths used to stop spread of pathogens from one area to another, need further consideration. In these cases, a Phase 3 test should be established to ascertain length of use of such baths both with respect to time period as well as frequency of use, for example how many boots can be disinfected in a boot bath and still ensure adequate disinfectant efficacy.

Norwegian authorities have suggested minimum levels of disinfectant use for “effluent water” from aquaculture. At present there is no CEN standard for assessing disinfection of water with high soiling conditions within the veterinary area. Currently, disinfection of water under the BPR is required for drinking waters. An OECD standard /ENV/JM/MONO(2012)15) exists for testing of bathing pools and spas. In the future, they may be adapted for used water from land based aquaculture and slaughter water emissions. Table 10 summarize relevant CEN standard applicable for different use categories. Where there are no test standards at present, we have indicated the need for such standards. Both mandatory (basic requirement) and optional (according to the claim) CEN standard tests are listed in the Table.

Table 10: Standard test methods for substantiating product claims. ND= no tests have been developed. DVG= Guidelines published by the German Veterinary Medical Society.

Type of activity	Test Phase, step	Product claim/Target sites							
		Water		Surface				Textile	Roe
		Intake water	Effluent water	porous	Non-porous	Instrument/ equipment	boot		
Bactericidal	2,1	EN 1276 modified[ab]		EN 1656					
	2,2	[c]	[c]	EN 16437	EN 14349			EN 16616 ASTM E24062	EN 16437
Yeasticidal	2,1	[a]	[b]	EN 1657					
	2,2	ND		DVG[d]	EN 16438			EN 16616 ASTM E24062	DVG[d]
Fungal spores	2,1	[a]	[b]	EN 1657					
	2,2	ND		DVG[d]	EN 16438			EN 16616 ASTM E24062	DVG[d]
Mycobacteria	2,1	[a]	[b]	EN 14204					
	2,2	ND		DVG[e]		ND	ND	EN 16616 ASTM E24062	DVG[e]
Bacterial spores		[a]	[b]	EN 13704		ND	ND		EN 13704
Virucidal	2,1	EN 14476 modified[ab]		EN 14675					
	2,2	[c]	[c]	DVG[f]	EN 17122			ASTM E2406	DVG[f]
Parasiticidal	2,1	[a]	[b]	DVG[g]			ND	DVG[g]	DVG[g]
	2,2	ND		DVG[g]			ND	ND	DVG[g]

[a]A standard test is needed that ensures that treated water is devoid of organisms that can cause harm to aquaculture organisms.
[b]A standard test is needed to ensure that spill water from aquaculture activities does not contain pathogen organisms that can spread into the natural environment.
[c] A simulated-use test should be proposed in agreement with the CA. [d]DVG guideline for fungicidal efficacy; [e]DVG guideline for tuberculocidal efficacy; [f]DVG guideline for virucidal efficacy; [g]DVG guideline for antiparasitic efficacy. Available at http://www.desinfektion-dvg.de/index.php?id=2219

4.4 Outline structure of the Aquadisinfectants Guidance Document

The Aquadisinfectants Guidance Document - A Guidance Document on the Efficacy testing of disinfectants used in Aquaculture in the EU and EEA, is a standalone guidance document aimed at supporting procedures for performance of efficacy testing of disinfectants intended to be used in aquaculture. It was created based on the premise that efficacy testing requirements in the current ECHA GD may not cover the broad range of disinfectants application within Norway and EU aquaculture.

The Aquadisinfectants Guidance Document (GD) was elaborated by comparing methods that are currently required for efficacy testing of disinfectants described in the ECHA GD, with the information stated in Sections 3, 4 and 5 (Results, Discussion and Conclusion) of the present report. A decision tree, as shown in Figure 1, was followed to guide decision on efficacy testing recommendations for each use pattern and target site considered relevant for the purpose of the Aquadisinfectant GD. Specifications of each step in the decision tree are explained in detail in Appendix 1, in which the target site "salmonid roe" is presented as an example.

Recommendations in the Aquadisinfectants GD are mostly based on salmonid aquaculture as more robust data were found for this culture. Furthermore, the anadromous nature of salmons cover applications in both fresh, brackish and sea waters. In addition, aquaculture of invertebrates such as mollusks and crustaceans tend to require less disinfection measures as they are practiced in open areas rather than closed systems. So that the use of disinfectants in fin fish aquaculture seems more relevant.

Following the findings stated in the present report, the Aquadisinfectants GD have the premise that efficacy testing should be performed under environmental conditions to which the disinfection product is claimed, including the worst-case conditions. The reasoning is that aquaculture is practiced in different European countries, and even regions within these countries, may present variable environmental conditions, e.g., temperature, water hardness and salinity.

As for the test organisms, this study highlighted the lack of information on published efficacy data of disinfectants, particularly in tests performed with standard species. This put in jeopardy clear conclusions concerning the choice of representative test organisms for testing efficacy of disinfectants intended to be used in Aquaculture. Therefore, until more studies are conducted, or specific standard guidelines are developed, the Aquadisinfectants GD recommends the use of current standard species, as described in Section 4.2 of this report. When exceptions apply, they are described and justified in the Aquadisinfectants GD.

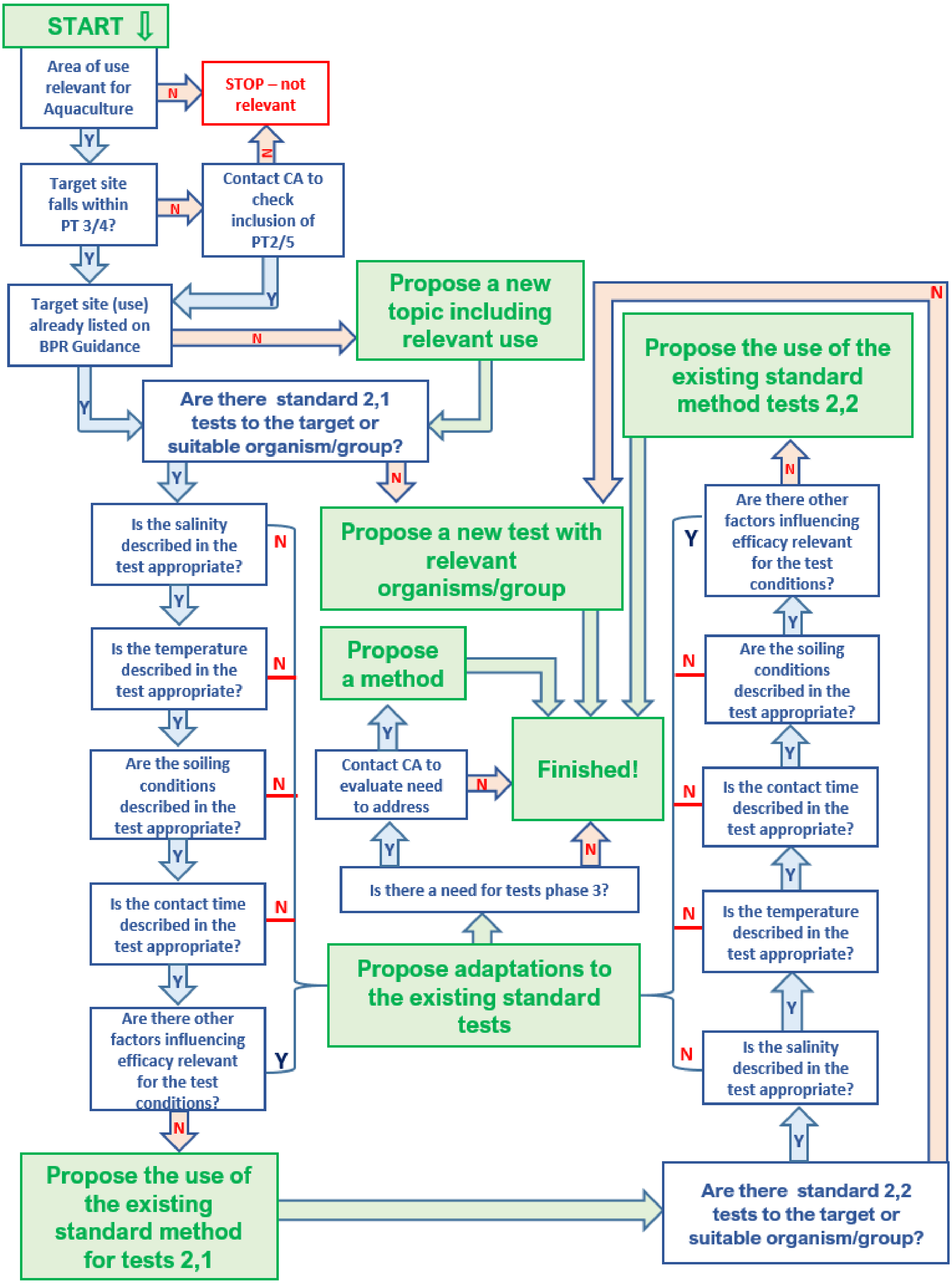

Figure 1. Decision tree showing the conditional pathways that were followed to decide on appropriate test requirements to meet relevant conditions for the use of disinfectants in Aquaculture.
CA = Competent Authority

The standard test organisms are described in CEN standard test protocols. The Aquadisinfectants GD recommend the use of the CEN standard test protocols as a general rule, and suggest modifications when relevant. The CEN standard guidelines are recommended according to the target site(s) of the disinfection product and comply with the requirements stated in the ECHA guidance document, as shown in Table 12.

Table 12. Equivalency between relevant target sites for Aquaculture and the use patterns described in the ECHA GD and, substantiating the choice of recommended CEN standard methods for aquaculture.

Target site in aquaculture	Equivalent Use patterns in the ECHA GD, from which the EN standards are recommended on the Aquadisinfectants GD
Water	PT5 – drinking water supplies and distribution systems PT5 – water in reservoirs
Surfaces	PT2 – biofilms PT3 – hard surfaces, hard surfaces in transport vehicles, boot baths PT4 – hard surfaces in food and feed areas, inner surfaces, equipment disinfection by soaking
Textile	PT 3 – textile disinfection
Roe	PT 3 – hatching eggs

See Appendix 1 for a detailed description of the methodology used for the Aquadisinfectants GD elaboration, following the decision tree as presented in Figure 1.

5 Conclusion

Information on relevant conditions for disinfection practices in the aquaculture industry was used to make a recommended guidance document on how to perform efficacy testing of disinfectants for use in the EU and EEA in accordance with the BPR. Most of the information on use and disinfection practices was collected from Norwegian aquaculture, and especially the salmonid industry has been used as an example to identify the different use patterns and disinfection practices. However, information about other aquaculture species and practices from other European countries are included in the assessments to make a guidance document that will be relevant for the EU BPR.

This guidance document is based on the authors best knowledge as described in this report. However, there are some knowledge gaps and limitations identified by the authors in this report:
There are limited data available for comparing aquaculture pathogens to standard test organisms regarding their sensitivity to active ingredients in disinfectants. The list of aquaculture pathogens in this report is not a comprehensive list of all relevant pathogens in Europe but intended to cover the most economically important. Thus, if new evidence is provided on aquaculture pathogens that are more robust and resistant to disinfectants than the standard test organisms, a re-evaluation of recommended test organisms for efficacy of disinfectants for aquaculture should be performed.

Aquaculture in Europe include a lot of different species, productions systems and practices which would require different use and targets for disinfectants. This report is limited to the use patterns or scenarios based on our own experience and from consulting farmers and disinfectant producers, mostly in Norwegian aquaculture but also in other European countries, and from reviewing the Norwegian fish health report and regulations by the Food Authorities. Details about the different conditions were limited to the main aquaculture species produced in Europe due to time and a lack of information being easily accessible from other (non salmonid) aquaculture industries outside of Norway.

Finally, there are currently no CEN guidelines for testing the efficacy of products for water disinfection in aquaculture. Therefore, until such guidelines exist, it is recommended that existing CEN guidelines, that have been developed for drinking water testing, are used for testing the disinfection efficacy in aquaculture.

6 References

1. ECHA 2022. Guidance on the BPR: Volume II Efficacy, Assessment + Evaluation (Parts B+C).
2. European Commission 2021. Strategic guidelines for a more sustainable and competitive EU aquaculture for the period 2021 to 2030. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. 12.05.2021: eur-lex.europa.eu.
3. Mattilsynet/ Norwegian Food Safety Authorothy 2022. Godkjente desinfeksjonsmidler i akvakultur / Approved disinfectants for aquaculture. https://www.mattilsynet.no/fisk_og_akvakultur/akvakultur/desinfeksjon/godkjente_desinfeksjonsmidler_i_akvakultur.802 (accessed 16.11.2022).
4. The European Parliament And The Council Of The European Union 2016. Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law') (Text with EEA relevance). In: EU, editor. Document 32016R0429. online: https://eur-lex.europa.eu/; 2016.
5. World Organisation for Animal Health (OIE) 2022. Aquatic Animal Health Code Chapter 1.3 Diseases listed by OIE. https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/?id=169&L=1&htmfile=chapitre_diseases_listed.htm (accessed 16.11.2022).
6. Eurostat 2022. Aquaculture production by species (fish_aq). https://ec.europa.eu/eurostat/cache/metadata/en/fish_aq_esms.htm (accessed 11.10.2022).
7. European Commission 2022. Overview of EU aquaculture (fish farming). https://oceans-and-fisheries.ec.europa.eu/ocean/blue-economy/aquaculture/overview-eu-aquaculture-fish-farming_en (accessed 08.11.2022).
8. Eurostat 2018. Aquaculture Statistics Handbook. https://ec.europa.eu/eurostat/cache/metadata/Annexes/fish_aq_esms_an2.pdf.
9. Miljødirektoratet/ Enviornmental Agency Norway 2022. Overgangsbestemmelser for biocidprodukter / National transitional requirements for biocides. https://www.miljodirektoratet.no/ansvarsomrader/kjemikalier/biocider/overgangsbestemmelser-for-biocidprodukter/ (accessed 05.09.2022).
10. Mattilsynet. Desinfeksjon av vann i akvakultur / Disinfection of water for aquaculture. 2020. https://www.mattilsynet.no/fisk_og_akvakultur/akvakultur/desinfeksjon/desinfeksjon_av_vann_i_akvakultur.3973 (accessed 05.09.2022.
11. Mattilsynet/ Norwegian Food Safety Authorothy 2013. Desinfeksjon av rogn / Disinfection of roe. https://www.mattilsynet.no/fisk_og_akvakultur/akvakultur/desinfeksjon/desinfeksjon_av_rogn.3972 (accessed 24.10.2022).
12. Veterinærinstituttet / Norwegian veterinary institute 2022. Veileder - Desinfeksjon av vann i akvakultur / Guidance document - Disinfection of water for aquaculture. https://www.vetinst.no/fagomrader/desinfeksjon/veileder-metoder-godkjent-for-desinfeksjon-av-vann-til-fra-akvakulturrelatert-virksomhet.
13. Mattilsynet/ Norwegian Food Safety Authorothy 2022. Brønnbåt og annen transport / Well boat and other transport. https://www.mattilsynet.no/fisk_og_akvakultur/akvakultur/bronnbat/ (accessed 26.10.2022).

14. Code of Good Practice 2022. Welcome to the Code of Good Practice for Scottish Finfish Aquaculture (CoGP). https://www.salmonscotland.co.uk/code-of-good-practice (accessed 26.10.2022).
15. Lazado CC, Good C 2021. Survey findings of disinfection strategies at selected Norwegian and North American land-based RAS facilities: A comparative insight. *Aquaculture* 2021; **532**: 736038.
16. Norwegian regulation FOR FOR-1997-02-20-192. Forskrift om desinfeksjon av inntaksvann til og avløpsvann fra akvakulturrelatert virksomhet. / Regulation on disifection of intake water to and discharge water from aquaculture activities. Online: Lovdata.
17. Sommerset I, Walde CS, Bang Jensen B, et al. 2022. Fiskehelserapporten 2021 / Fish health report 2021: Veterinary Insitute Norway.
18. Majeed M, Saleh M, Menanteau-Ledouble S, Palić D, El-Matbouli M. 2018. Dissemination, identification and treatment of Aphanomyces invadans, the causative agent of epizootic ulcerative syndrome in fish. *Wien Tierarztl Monatsschr*; **105**: 63-71.
19. Graham D, Cherry K, Wilson C, Rowley H 2007. Susceptibility of salmonid alphavirus to a range of chemical disinfectants. *Journal of fish diseases*; **30**(5): 269-77.
20. Verner–Jeffreys DW, Joiner CL, Bagwell NJ, Reese RA, Husby A, Dixon PF 2009. Development of bactericidal and virucidal testing standards for aquaculture disinfectants. *Aquaculture*; **286**(3-4): 190-7.
21. Hernndez A, Martro E, Matas L, Martın M, Ausina V 2000. Assessment of in-vitro efficacy of 1% Virkon® against bacteria, fungi, viruses and spores by means of AFNOR guidelines. *Journal of Hospital Infection*; **46**(3): 203-9.
22. Smail D, Grant R, Simpson D, Bain N, Hastings T 2004. Disinfectants against cultured Infectious Salmon Anaemia (ISA) virus: the virucidal effect of three iodophors, chloramine T, chlorine dioxide and peracetic acid/hydrogen peroxide/acetic acid mixture. *Aquaculture*; **240**(1-4): 29-38.
23. Martin H, Soumet C, Fresnel R, et al. 2013. Comparison of the virucidal efficiency of peracetic acid, potassium monopersulfate and sodium hypochlorite on hepatitis A and enteric cytopathogenic bovine orphan virus. *Journal of applied microbiology* ; **115**(4): 955-68.
24. Mainous ME, Kuhn DD, Smith SA 2011. Efficacy of Common Aquaculture Compounds for Disinfection of Aeromonas hydrophila, A. salmonicida subsp. salmonicida, and A. salmonicida subsp. achromogenes at Various Temperatures. *North American Journal of Aquaculture* 2011; **73**(4): 456-61.
25. Meinelt T, Phan T-M, Behrens S, et al. 2015. Growth inhibition of Aeromonas salmonicida and Yersinia ruckeri by disinfectants containing peracetic acid. *Diseases of aquatic organisms*; **113**(3): 207-13.
26. Wedemeyer GA, Nelson NC 1977. Survival of two bacterial fish pathogens (Aeromonas salmonicida and the enteric redmouth bacterium) in ozonated, chlorinated, and untreated waters. *Journal of the Fisheries Board of Canada*; **34**(3): 429-32.
27. Johnsen BO, Ugedal O 2001. Soppinfeksjoner (Saprolegnia spp.) på laksefisk i Norge-statusrapport. NINA Oppdragsmelding 716:1-34. ISBN 82-426-1268-4
28. Evans Vanodine International 2022. Buffodine Microbiological Profile. https://www.evansvanodine.co.uk/assets/Buffodine-is.pdf
29. Benchmark Genetics 2019. A5 Atlantic Salmon Hatchery Manual V1/6 August 2019/GEN-NO-10032. https://wp-bmkgenetics-2022.s3.eu-west-2.amazonaws.com/media/2021/05/01133146/Atlantic-Salmon-Hatchery-Manual_Norwegian.pdf

Appendix 1

Appendix 1 explains in detail how the decision tree (shown in Section 4.4, Figure 1) was followed to conclude on recommendations on the test methods and target organisms for efficacy testing of disinfectants intended to be used in aquaculture in EU and EEA.

Here, the example described led to decisions concerning the choice for testing efficacy of disinfectants against bacteria in disinfection products intended to be used on salmonid roe. In this example, the disinfectant Buffodine was used.

Applying the decision tree, the first question is if the **Area of Use** is relevant for Aquaculture. As newly stripped and eyed fish eggs must be disinfected in hatcheries, this is relevant, and we move forward on the decision tree. The next question is whether this area of use falls within one of the **PT concerning disinfectants**. The disinfection of hatching-eggs is considered under PT3, topic 5.4.3.7 (disinfection of hatching-eggs as **target sites**) therefore the next step of the decision tree is to check if there are already standard tests for the **phase 2,1** of efficacy test. The Annex 4 of the ECHA GD lists the following **EN standard** as the guidelines to be followed for efficacy testing in the phase 2,1: EN 1656 for bacteria and EN 1657 for fungal spores. The next step is to go through these standard guidelines and check if they are robust enough to cover the range of microorganisms that need to be addressed as well all the most important environmental conditions in Aquaculture in Norway and EU. To perform this task, we compared test conditions and standard organisms recommended in the CEN guidelines with the tests that are required by the Norwegian legislation for Aquaculture and used the active ingredients/products authorized for marketing in Norway as examples of desired conditions.

For purposes of simplicity, the example provided will focus on the bactericidal activity only.

The example below compares the characteristics and use conditions of the disinfectant Buffodine, authorized for the disinfection of salmonid eggs in Norway, with the use conditions of a generic disinfecting product aimed to be used similarly (disinfection of hatching eggs) according to the requirements stated in the ECHA GD, Section 5.4.3.7 and Appendix 4.

Table A1. Bactericidal activity and conditions of the disinfectant Buffodine

20BACTERIAL TEST PROFILE					
ORGANISMS	DILUTION	TEST METHOD	TEMP (°C)	CONTACT TIME (MINUTES)	SOILING LEVEL
Enterococcus hirae	1:100	EN 1656	10	30	Low
Proteus vulgaris	1:200				
Pseudomonas aeruginosa	1:25				
Staphylococcus aureus	1:50				
Aeromonas salmonicida	1:50		4		High
			20		
Carnobacterium maltaromaticum	1:10		4		
	1:20		20		
Lactococcus garvieae	Undiluted		4		
	1:10		20		
Yersina ruckeri	1:25		4		
	1:50		20		

Source: [28]

According to our research, relevant pathogens in the Norwegian Aquaculture scenario are listed in Section 3.5 of this report. Buffodine has showed efficacy against many of the most relevant bacteria in EU Aquaculture, namely *Aeromonas salmonicida* and *Yersina ruckeri*.

When comparing the bacteria listed as test organisms in the EN 1656 standard, which is the recommended guideline to test efficacy against bacteria, the list of bacteria that are used as target organisms in the tests does not include relevant pathogens in Aquaculture. Similarly, the target site "hatching eggs" is not described as an area of use in the NS-EN 14885:2022, which is a summary compilation of the current CEN protocols and was consulted in this exercise. However, the NS-EN 14885:2022 states that tests can be performed with any relevant bacteria for a given intended use in the veterinary area. In such cases, temperature, contact time and soiling conditions are not specified and can be adjusted as needed. Lg reduction should always be ≥5.

Despite there are no specific tests targeting the relevant microorganisms in Aquaculture, we have the opinion that the test **EN 1656 can be used as a starting point to evaluate the efficacy of hatching eggs disinfectants against bacterial activity**, in the phase 2, step 1. The reason is the lack of evidence, to date, that standard organisms are more (or less) tolerant to disinfectants than aquaculture pathogens (see report, Section 3.6). In addition, as the existing CEN protocols are flexible towards the inclusion of different test organisms under relevant conditions, relevant aquaculture pathogens might be used as test organisms if they become standard species in the future, or if such tests are agreed by the CA.

Efficacy against bacterial activity: Phase 2,1

Based on the information above, we will use the EN 1656 as a starting point to recommend the better conditions for testing the bactericidal efficacy of a product claiming authorization as a disinfectant of salmonid roe, at the stage 2,1 (suspension test) of the tiered approach.

Table A2. Modifications of test conditions on the EN 1656 to reflect aquaculture conditions

	EN 1656 Original	EN 1656 with modifications
Use Area	Surface disinfection	Aquaculture
Target site	hatching eggs	Freshly fertilized salmon roe in Norwegian hatcheries*
Test species	*Pseudomonas aeruginosa* ATCC 15442 *Proteus hauseri* ATCC 13315 *Staphylococcus aureus* ATCC 6538 *Enterococcus hirae* ATCC 10541	*Staphylococcus aureus* ATCC 6538 Test against other relevant bacteria might be added according to the claim
Salinity	freshwater	Freshwater, or as claimed if a different species
Temperature	30°C	10°C mandatory, others as claimed
pH	Same as the biocide product	Comfortable for the animal welfare
Contact time	As claimed	As claimed
Soiling conditions	Clean/dirty	As claimed
Pass criteria (lg reduction)	≥ 5	5
References	Standard Norge 2022; ECHA 2022	See [10,17,28,29]

*Can be also applied to other fresh water and even marine species, by adapting the method to reach relevant test conditions in each case.

Efficacy against bacterial activity: Phase 2,2

The tiered approach requires that a quantitative carrier test (phase 2, step 2) is performed in addition to the suspension test presented above (Phase 2,1). The product can be authorised only if it passes the efficacy criteria of both tests for the intended use.

The same approach used to define and justify the modifications above (phase 2,1) was applied here. The EN 16437 protocol is used to evaluate efficacy against bacteria in the phase 2,2. Table A3 shows the current conditions of the test EN 16437 as well as the suggested modifications.

Table A3. Modifications of test conditions on the EN 16437 to reflect aquaculture conditions

	EN 16437 Original	EN 16437 with modifications
Use Area	Surface disinfection	Aquaculture
Target site	hatching eggs	Freshly fertilized salmon roe in Norwegian hatcheries*
Test species	*Pseudomonas aeruginosa* ATCC 15442 *Proteus hauseri* ATCC 13315 *Staphylococcus aureus* ATCC 6538 *Enterococcus hirae* ATCC 10541	*Staphylococcus aureus* ATCC 6538 Test against other relevant bacteria might be added according to the claim
Salinity	freshwater	Freshwater, or as claimed if a different species
Temperature	10°C - also permitted 4, 20, 40	10°C mandatory, others as claimed
pH		Comfortable for the animal welfare
Contact time	Any of 1, 5, 15, 30, 60 min and then at 30 min intervals up to 360 min	As claimed
Soiling conditions	Clean. Permit the use of any relevant substance	As claimed
Pass criteria (lg reduction)	≥ 4	4
References	Standard Norge 2022; ECHA 2022	See [10,17,28,29]

*it can be also applied to other fresh water and even marine species, by adapting the method to reach relevant test conditions in each case.

Justification for the modifications suggested (EN 1656 and EN 16437):

- Target site: nomenclature changed to reflect the specificity of the product (salmonid eggs).
- Test species: until further evidence, testing using one gram-negative species is sufficient. *S. aureous* is the standard bacteria recommended by the Norwegian Food Safety Authority (NFSA)
- Temperature: Salmonid eggs develop in cold waters, therefore lower test temperatures are required
- pH: as recommended by NFSA

Conclusion and recommendations

By applying the decision tree and based on the information provided in the report, we have the opinion that the existing EN standard methods EN 1656 and EN 16437 should be recommended for testing the efficacy of disinfection products against bacteria. Test conditions should be modified or adapted to reflect relevant environmental conditions in which aquaculture is practiced in Norway and EU.

Final considerations

This Appendix brings one example on how decisions concerning recommendations in the Aquadisinfectants GD are provided. The same approach, of using the decision tree and information presented in this report, was used for all use patterns and target sites presented in the Aquadisinfectants GD.

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