National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Knowledge brief

Should biocides that control *Legionella* (*pneumophila*) in engineered water systems be effective against biofilm?

RIVM

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Questions and answers

On November 20th 2024 the National Institute for Public Health and the Environment (RIVM) received the following questions from the Dutch Board for the Authorisation of Plant Protection Products and Biocides (Ctgb):

1. Can the control of *Legionella* bacteria in the water phase be seen separately from the control of the biofilm?
2. If so, does it comply with the Dutch policy regarding *Legionella* control to allow biocides that only control *Legionella* bacteria in the water phase and not the biofilm?
3. If not, how or where is it stated that both the water phase and the biofilm phase should always be tackled when controlling *Legionella*?

The answers to the questions are:

1. No, to control *Legionella (pneumophila)* in water systems you also need to control (the amplification of) *Legionella* in biofilm. In water systems *Legionella* bacteria are present in biofilm and use amoebae and other protozoa that 'graze' this biofilm for amplification. *Legionella* bacteria also use the biofilm and amoebae to protect themselves from stress factors like biocides. If the *Legionella* in biofilm and amoebae are not controlled these bacteria will stay present in the water system, multiply and, if aerosolizing is possible, will be dispersed in the air.
2. No, see answer 3.
3. The current Dutch legislation does not always explicitly state that both the water phase and biofilm present in the water system should be controlled. However, the legislation states that a risk assessment and management plan must be made for the entire water system. This includes both the water phase and the installation with biofilm. For example, the legislation for premise plumbing systems and cooling towers states that the risk assessment has to address the possibility of biofilm being present and, for cooling towers, if the water treatment program is effective against the formation of biofilm. Legislation also states that the management plan must have measures that control amplification of *Legionella*. In order to control amplification you need to control the presence of amoebae (in biofilm).

References and further explanation are given below.

Main references

The main reference for the answers above is the report 'Management of Legionella in Water Systems' of the National Academies of Sciences, Engineering and Medicine (NASEM, 2020). This comprehensive report gives an overview of the current knowledge about *Legionella* and how to control *Legionella* in water systems. Another main reference is the recently published RIVM report 'Effectiveness and considerations of *Legionella* control methods applied in premise plumbing systems' (Bartels et al., 2024; in Dutch).

Biofilm and protozoa: growth and protection

Legionella (pneumophila) in water systems (e.g. cooling towers and premise plumbing systems) need protozoa and biofilm for amplification (NASEM, 2020; Bartels et al., 2024). NASEM (2020) states that primary growth in engineered water systems is within amoebae or other free-living protozoa that are associated with biofilms (see page 50-51).

Pathogenic *Legionella* bacteria are able to replicate within these protozoa to problematic levels (see figure 1). The RIVM report (Bartels et al., 2024) describes that *Legionella* are mainly associated with biofilm and use protozoa like amoebae for amplification. This is also consistent with recent studies and reviews (Ashbolt, 2023; Cavallaro et al., 2023; Margot et al., 2024; LeChevallier et al., 2024; Ortlieb et al., 2024). Biofilm and protozoa can also protect *Legionella* from biocides (NASEM, 2020; Bartels et al., 2024). Amoebae can create a cyst to protect themselves against stress factors like heat or chemicals (NASEM, 2020; Bartels et al., 2024). *Legionella* inside these amoebae cysts are preserved and able to multiply again after the cyst phase (NASEM, 2020; Ashbolt, 2023).

Biofilm versus water phase

In water systems the natural habitat of *Legionella* is the biofilm. However, biofilm material is regularly released into the water phase. NASEM (2020) described this process as follows: '(…) large numbers of *L. pneumophila* can be released from the biofilm environment within fragments of biofilm, within protozoan trophozoites and cysts, or within expelled amoebal-vesicles (membrane-bound structures containing undigested food and microorganims). These released *L. pneumophila* cells then disperse in the bulk water phase among other planktonic microorganisms.'

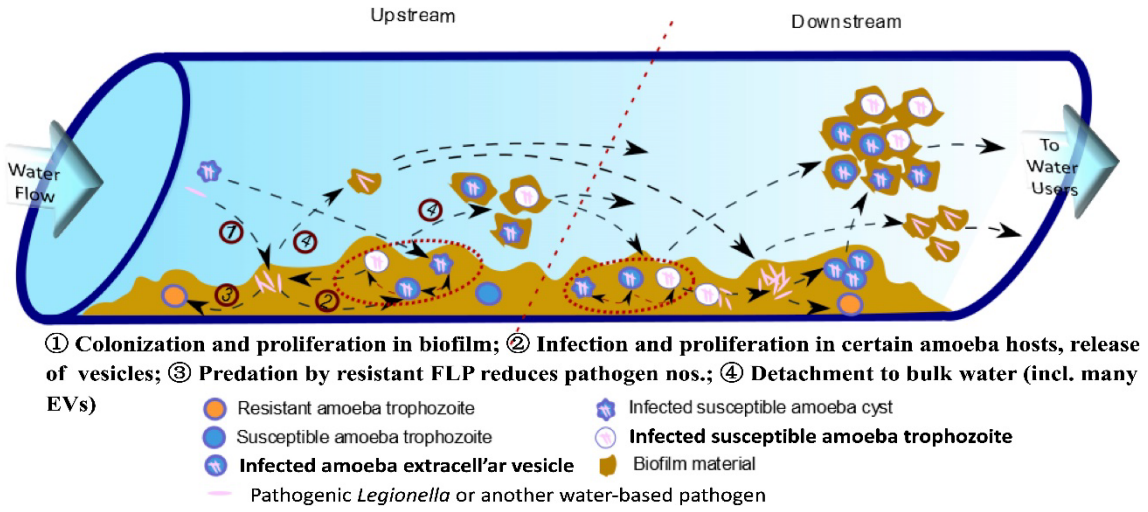

Figure 1 Conceptual model for explosive growth of Legionella in engineered water system biofilms. FLP - free-living protozoa (primarily amoebae and ciliates) grow on biofilm constituents, ultimately limited to feeding on less-preferred amoeba-resisting bacterial, such as L. pneumophila, leading to explosive growth and release in extracellular amoebal vesicles (EVs) that may enhance downstream amoebal predation over less preferred free bacteria – so increasing proliferation of pathogens. Fragments of biofilm with Legionella and amoeba (vesicles) are regularly released in the water phase. Source: Ashbolt (2023).

The presence of *Legionella* in the water phase after release from the biofilm can potentially be very short (seconds or less than a minute). Released biofilm material can be carried by the (continuous) water flow directly to the aerosol producing part of the water system (e.g. shower head or cooling tower nozzles). Exposure to *Legionella* has been linked to the use of shower hoses and heads (Whiley et al., 2015; Proctor et al., 2017; Hayes-Phillips et al., 2019; Cavallaro et al., 2023). If there is stagnation, *Legionella* can be present in the water phase for longer.
The biofilm in the water system can be seen as the reservoir. From this reservoir fragments of biofilm with *Legionella* bacteria and amoebae or amoebal-vesicles containing *Legionella* are released into the water phase (see figure 1). The concentration of *Legionella* in the water phase can differ depending on the situation (biofilm composition, change of water flow and/or temperature, pipe material used and age, disinfection method used, etc.).

Biocides as a control strategy for *Legionella*

Biocides are used to either control growth or eradicate *Legionella* in engineered water systems. To eradicate all pathogenic *Legionella* in a water system, use of a temporarily high concentration of biocides is necessary to inactivate all *Legionella* in amoebae and biofilm (NASEM, 2020; Bartels et al., 2024). Biocides used to continuously control growth in water systems should at least limit growth in amoebae and biofilm as much as possible in order to limit the dispersion into the water phase (NASEM, 2020; Bartels et al., 2024). Even if the focus is only on the water phase a biocide has to be effective against biofilm material consisting of *Legionella* within amoebal-vesicles because this material can be present in the water phase and lead to exposure. Also, biocides that are only effective in the water phase should be able to inactivate *Legionella* in seconds or at least in less than half a minute because the time between biofilm release and the aerosolizing part of the water system could be seconds.

Dutch legislation for *Legionella* control in biofilm and protozoa

Dutch legislation for the prevention of Legionnaires' disease is largely based on controlling growth of *Legionella* in engineered water systems. For premise plumbing systems of priority locations, 'bathwater basins' (e.g. hot tubs and swimming pools) and wet cooling towers a risk analysis and management plan is needed if aerosolization into the air is possible ('Drinking Water Decree' and 'Environmental Activities Decree' (Bal) paragraph 4.46 and chapter 15; both in Dutch only). For premise plumbing systems and bathwater basins additional actions are necessary if 100 cfu/L or more *Legionella* is detected in a water sample. Aerosolizing systems used during work also must contain less than 100 cfu/L *Legionella* in order to prevent exposure for employees ('Working Conditions Decree', article 4.87b; in Dutch only).

Assessing whether biofilm (formation) is present in premise plumbing systems or wet cooling towers is part of the risk assessment. In appendix 2, article 5.1.1. of the 'Regeling legionellapreventie in drinkwater en warm tapwater' (regulations that are part of the Drinking Water Decree) it is stated that you have to take risk factors into account that can enhance amplification of *Legionella,* such as biofilm and sediment ("Bij de risicoanalyse wordt tenminste rekening gehouden met de volgende risicofactoren die vermeerdering van legionellabacteriën in drinkwater- of warm tapwaterinstallaties bevorderen: (…) d. biofilm en sediment"). For wet cooling towers it is necessary to assess if the water treatment program is effective against the formation of biofilm ("Bij het onderzoek worden in ieder geval betrokken: (…) c. de effectiviteit van het waterbehandelingsprogramma voor legionellabacteriën en biofilmvorming;"; Bal, artikel

4.570, lid 2c). Legislation also states that the management plan must include measures that keep the wet cooling tower clean as well as the water in it ("Het schoonhouden van de natte koeltoren en het water dat zich daarin bevindt"; Bal, artikel 4.571, lid 1f3). Measures are also needed to prevent amplification ("het zoveel mogelijk beperken van de vermeerdering van legionellabacteriën door toepassing van waterbehandelingstechnieken"; Bal, artikel 4.571, lid 1f4). In order to comply with these rules biocides need to be effective against protozoa (amoebae) and biofilm.

This knowledge brief was written on behalf of the Ctgb within the project Policy advice and methodology for plant protection products and biocides of the Ministry of Infrastructure and Water Management.

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