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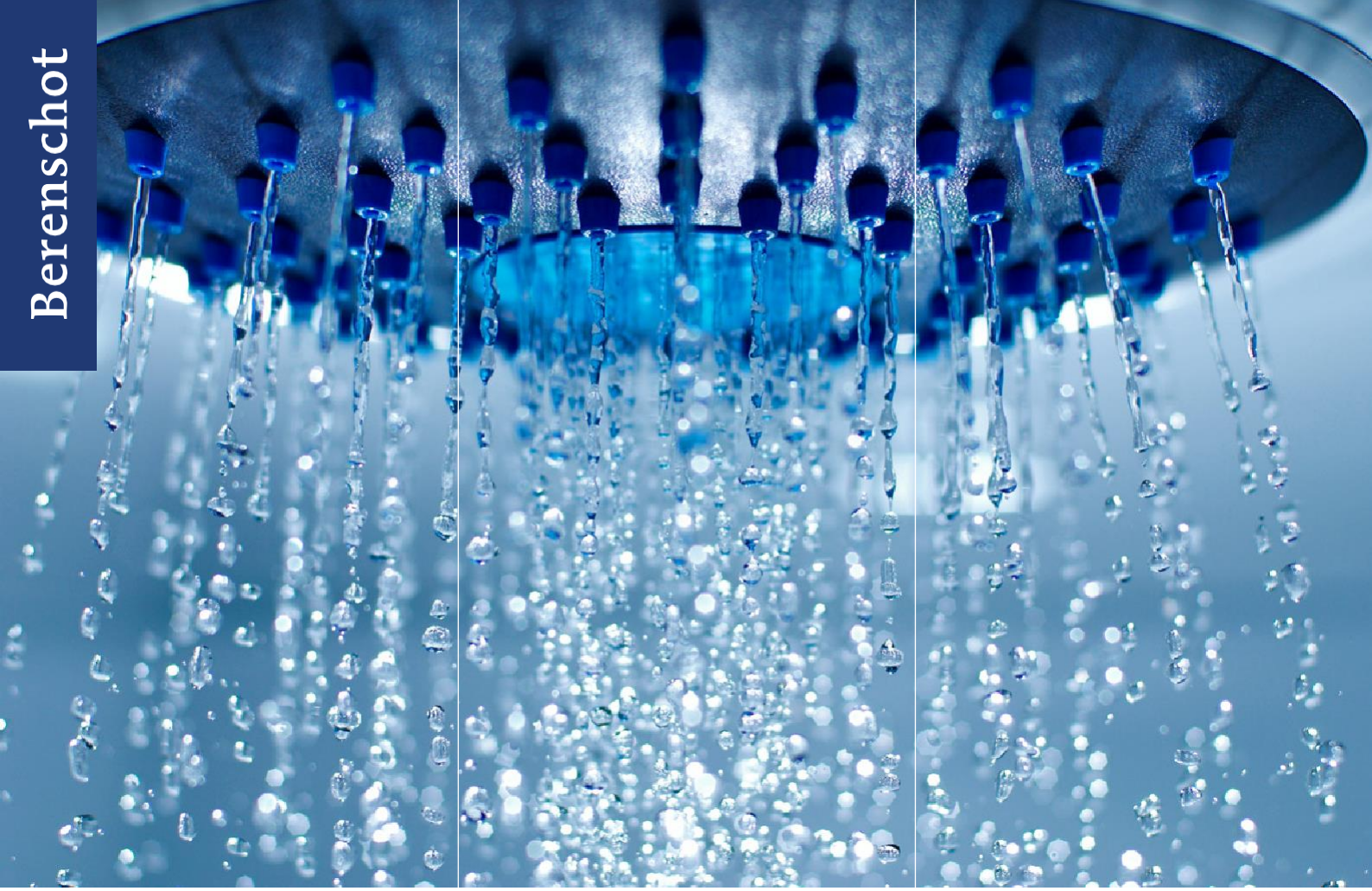

Rightly to a more effective legionella prevention

A forward-looking review of regulations on legionella prevention in tap water systems based on scientific and legal analysis

June 2, 2021

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A forward-looking review of the regulation of legionella prevention in tap water systems based on scientific and legal analysis

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CHAPTER 1

The review of legionella regulation

1.1 Context and reason

Legionella is a bacterium, which ISSO Publication 55.1 states can grow in water with a temperature between the 25 and 50°C range. *Legionella* is capable of propagating in a wide range of water systems, including cooling water systems, wastewater treatment systems, fountains, process water systems, swimming pools, and tap water systems.

The growth of *Legionella* in water systems potentially poses a threat to public health. When people inhale a pathogenic strain of Legionella bacteria, there is a chance of Legionnaires' disease (Legionellosis), which can range from flu-like symptoms to severe pneumonia (Legionnaires' disease). Because the growth of pathogenic legionella species can occur in tap water and thus pose a threat to the public health, legislation is in place in the Netherlands aimed at controlling the growth of pathogenic legionella species in tap water systems.

The immediate reason for drafting rules on the prevention of *Legionella* in tap water systems lies in the Legionella outbreak in Bovenkarspel in 1999.
The main objective of the legislative framework is to prevent Legionellosis and not to achieve complete absence of *Legionella* in tap water systems. In connection with an increased risk for vulnerable people to become infected with pathogenic legionella species, certain categories of tap water installations receive special regulatory attention. These are the so-called priority establishments, such as hospitals, healthcare facilities, lodging houses, swimming pools, camping sites, marinas, truck stops, asylum seeker centers and prisons.

The manager of such a location1 is obliged to meet a quality requirement for *Legionella* and also to test through a risk analysis whether it can be met. If a risk is demonstrated, there is an obligation to draw up a Legionella management plan and to implement measures and controls in accordance with that management plan.

The current legislative framework still builds on insights from 2000. Since that time, much research has been done on Legionella. In the meantime, the scientific view on the growth of *Legionella* in tap water systems and the effectiveness of control measures (such as flushing with hot water) has changed. There are also signals that the feasibility and enforceability of the rules are under pressure. With this, stakeholders are questioning the effectiveness of the current legislative framework. In order to create regulations that set requirements that actually lead to the prevention of legionellosis, it is important to regularly check whether there are any new scientific insights that require adjustment of the regulations. This report addresses this issue.

1.2 Purpose and question

In light of the above, there is a need for an evaluation of the current regulations for legionella prevention in tap water systems. The purpose of the evaluation is to identify whether the current scientific understanding of legionella prevention gives cause to change the regulations and, if so, how the content of the applicable regulations should be modified.

1 In accordance with the Drinking Water Decree, this also applies to an owner of a collection- ve water supply to which taps are directly or indirectly connected.

The central research question of this evaluation thus reads as follows:

Central Research Question

Based on current scientific knowledge, which regulations should be modified, and in what way?

Answering the central research question leads to the following three sub-questions:

1. What current insights on legionella prevention prompt changes to existing regulations and why?
2. What are strengths and weaknesses in theory and practice in current regulations and why?
3. How can the existing regulations on legionella prevention be substantively modified?

Scope

This study focuses exclusively on legionella prevention in drinking water and hot tap water systems. The scientific knowledge gathered and the regulatory framework set out therefore relate exclusively to these systems. Within these systems, seven themes have been identified for this research. The themes were determined in agreement with the supervisory committee for the evaluation2 . The seven themes are as follows:

- **The effectiveness of thermal management (and related risk qualification).**
- **Occurrence of *Legionella* in cold water versus hot water systems.**
- **The influence of (weekly) flushing of tap water installations on *Legionella* in buildings.**
- **The influence of material use in tap water installations.**
- **Monitoring of *Legionella* spp, culturable *Legionella* spp and/or *Legionella pneumophila*.**
- **The risk of a volume less than one liter.**
- **The risk qualification for collective water supply or piping system.**

For these themes, the scientific insights were examined and it was indicated to what extent these insights give cause to adjust the current regulations.

Based on the terms of reference, this study does not focus on the effectiveness of management measures other than those mentioned above in the report. This means that this report also does not make a statement about the effectiveness of other legionella management versus thermal legionella management.

The research questions to answer the central question and the three sub-questions we operationalized in the evaluation framework. The framework shows how we answered the central question and underlying sub-questions. The evaluation framework is attached in Appendix 2.

1.3 Research Justification

This research had a scientific phase and a managerial-legal phase. In the scientific phase, we reviewed and described recent scientific research concerning the seven central themes. Based on the scientific analysis, regulatory adjustments were identified. The managerial-legal phase consisted of testing the identified adjustments and deepening the meaning of the changes in practice. Based on the insights from both phases, we arrived at an answer to the central research question and resulting conclusions and recommendations.

We discussed the progress of the study with the supervisory committee in the interim (for a list of supervisory committee participants, see Appendix 3).
The topics of the study were coordinated with the supervisory committee, an interim report was discussed upon completion of the scientific phase, and a draft final report was discussed upon completion of the managerial-legal phase.

Scientific Phase

The scientific phase of the study consisted of a literature review of recent scientific insights concerning the seven central themes identified in Section 1.2. The report *Management of Legionella in Water Systems* from the US National Academies of Sciences, Engineering, and Medicine and the references included therein were the starting point for the literature study.

2 More topics were mentioned by the supervisory committee, but it was agreed with the client that they would be addressed in a separate process.

In addition to the literature review, we conducted interviews with experts and professionals with the aim of testing and further enriching the scientific insights (for an overview of respondents see Appendix 3). The scientific phase thus resulted in an overview of the state of the art concerning the seven central themes as well as in a first set of possible adjustments concerning the laws and regulations concerning legionella prevention.

Managerial-legal phase

The administrative-legal phase focused on gaining insight into administrative and legal concerns that play a role in the design of laws and regulations. For this purpose, we first of all conducted interviews with various professionals in order to ascertain what concerns regarding feasibility, effectiveness and efficiency exist for the identified modifications for regulation based on the scientific insights. Also, during the administrative-legal phase, a collision test was organized with members of the National *Legionella* Platform (LOPL), with the aim of exchanging views on The meaning in practice of the identified changes in laws and regulations. The results from the interviews and We then analyzed the impact test and used it to come up with answers to the seven questions of the Integral Assessment Framework for Policy and Regulation (IAK). The IAK contains standards that good policy or regulation should meet. The answers to the seven IAK questions aimed to contribute to the design of effective regulations. The answering of these seven questions also led to the answering of the central main question of this study, which we have answered in a draft report. The draft report was discussed in a final collision test with the supervisory committee, after which we arrived at a final report.

1.4 Reader's Guide

In Chapter 2 we provide a scientific introduction to *Legionella* as well as a description of the regulatory framework for the rules on Legionella prevention in drinking water and hot water systems. We describe in which laws, The rules for legionella prevention are included in the General Administrative Orders (AMvBs), regulations and standards, and how they relate to each other. We also discuss the distinction between piping networks and installations in priority settings and pipeline networks and facilities outside the priority setting, and we name adjacent regulations.

In Chapters 3 through 9, we elaborate on the central topics for this study listed in Section 1.2 for each chapter. Each chapter starts with a brief overview of current legislation and an explanation of why the specific topic was included in this review. We then provide a brief overview of the state of the science for the topic at hand to 2001, which is followed by an overview of the scientific findings since 2001, which are then summarized in a section with the main conclusions. In order to keep facts and opinions separate, we have included below a separate section reflecting knowledge and practical experience from the interviews. We conclude each chapter with a preliminary recommendation on regulatory change for the topic at hand, based on the scientific insights.

In Chapter 10 we answer the main central question, the sub-questions and the questions from the evaluation framework. In line with the evaluation framework, the chapter starts by answering the IAK questions. We then use the ensuing shipping line to answer the central question and the sub-questions.

In Appendix 1, we have included a list of references to scientific studies that we used during this study. Appendix 2 contains the evaluation framework. In Appendix 3, we have included a list of respondents, members of the supervisory committee, and participants in the LOPL collision trial. Finally, Appendix 4 contains an overview of other bottlenecks experienced by the field.

B

CHAPTER 2

Scientific and legal introduction

In this section we introduce in a general sense Legionella and the growth of Legionella in drinking water. This section is not yet the inventory of current scientific knowledge in relation to regulations.
This follows in the following sections and is based on the analysis of about 190 different scientific publications. Most of the information we describe in this short section was also known before 2001 and we extracted it mainly from two literature reviews (van der Kooij, 2014, National Academy of Sciences, 2019).

2.1 Scientific introduction Legionella and drinking water

In recent years, the number of reported cases of Legionella pneumonia (also called Legionnaires' disease) in the Netherlands has increased to 550 to 600 per year. However, the number of people who become ill from *Legionella* is probably higher, due to underdiagnosis. For the Netherlands, it is estimated that the actual number of patients with Legionella pneumonia in the hospital is 1000 to 14,000. Worldwide, 90 to 98% of the reported cases of Legionella pneumonia caused by the bacterium *Legionella* pneumophila, which belongs to the genus Legionella. The genus *Legionella* contains more than sixty different described species and a large number of not (yet) isolated and described species. In addition to *L. pneumophila,* twenty other legionella species have been described as capable of causing disease. Most of the legionella species described are aquatic and reproduce in freshwater ecosystems, including several man-made freshwater systems (e.g., tap water systems, cooling towers, and wastewater treatment plants).

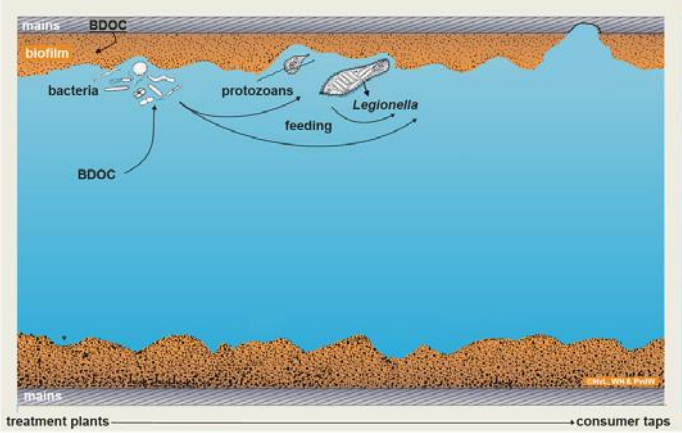


Figure 1. **Multiplication of Legionella in protozoa grazing on biofilm in drinking water systems. BDOC = biodegradable organic carbon.**

The drinking water produced and distributed by the Dutch drinking water companies always naturally contains bacteria belonging to the genus *Legionella,*
but these legionella populations in the clean water and drinking water distribution system are dominated by species that have not (yet) been isolated and described. These species are not likely to pose a risk to public health.

Multiplication of pathogenic legionella species in the drinking water ecosystem occurs primarily in tap water systems in buildings, by allowing the temperature there to be in the favorable range for growth of pathogenic *Legionella* (usually between 25 to 42°C). The propagation of *L. pneumophila* in the tap water systems of buildings has been best studied and has shown
that this propagation is a relatively complex ecological phenomenon (Figure 1). It follows from Figure 1 that propagation of *L. pneumophila* almost always occurs in protozoa, single-celled animal organisms. These protozoa graze in tap water systems particularly on biofilm that has developed on materials in contact with drinking water.
Normally, bacteria ingested by protozoa in this way are broken down by the protozoa, releasing energy that is used by the protozoa for growth. However, *L. pneumophila* is not broken down in certain protozoa (so-called host protozoa), but rather can multiply in these protozoa. Because of this multiplication of *L. pneumophila* in the protozoa, this protozoan cell can become so full of cells of *L. pneumophila*
that it snaps open, after which the released *L. pneumophila* cells can be taken up again by other host protozoa and the cycle repeats itself. Because of this growth mode of *L. pneumophila*, propagation of *L. pneumophila* is thus directly dependent on the presence of host protozoa and indirectly dependent on the biofilm concentration where
the protozoa graze on. Although the growth of other legionella species has not been as well studied as that for *L. pneumophila*, many other described
legionella species found that they can reproduce in protozoa.

The growth of *Legionella* is (in)directly dependent on a number of factors. For example, the concentration of nutrients, acidity (pH) and temperature are important factors that determine whether multiplication occurs. It has been observed that when the concentration of bacterially degradable organic substances in drinking water increases, the numbers of *Legionella* also increase. These degradable substances can be present in the drinking water or can come from plastic and/or rubber pipe material. It has also been seen that increased concentrations of iron stimulates the growth of at least *L. pneumophila*. Furthermore, the
acidity of drinking water always in the range for growth of the pathogenic legionella species.

Because the growth of these pathogenic legionella species can occur in drinking water and thus pose a threat to public health, legislation is in place in the Netherlands aimed at controlling the growth of pathogenic legionella species in tap water systems. The control
of legionella multiplication in tap water systems in the Netherlands is mainly achieved by keeping the temperature of the drinking water at most locations in the tap water system outside the range for growth of *L. pneumophila*. Specifically, this means trying to keep the cold tap water temperature below 25°C and the hot tap water temperature above 55°C. In addition, the preparation and implementation of a legionella management plan for tap water installations of priority institutions is an important cornerstone of the legionella regulations in the Netherlands.

Part of this Legionella Management Plan is the periodic checking of drinking water samples for the presence of Legionella. This monitoring focuses on Legionella species that can multiply on a selective agar medium as described in ISO 11731. In this ISO 11731
described three agar media, buffered charcoal yeast extraction (BCYE), BCYE with antibiotics (GVPC medium), and Modified Wadowksy Yee (MWY) agar, with the BCYE and GVPC agar media mostly used for samples with low concentrations of interfering flora (e.g., drinking water) and the MWY agar mostly used for samples with high concentrations of interfering flora (e.g., cooling tower water or wastewater). Because this report focuses on the evaluation of the legionella legislation of drinking water and hot tap water, the rest of the report uses the BCYE medium as term used for BCYE and GVPC medium, because both culture media use BCYE as a base.

Incidentally, it is known that a large proportion of Legionella species (not yet described) and some forms of the described Legionella species (the so-called 'viable but non-culturable' forms) do not multiply on BCYE or MWY agar medium. In this report, Legionella bacteria cultured on selective agar medium using the ISO 11731 method are referred to as culturable *Legionella* spp or culturable *L. pneumophila*. With
Legionella spp or *L. pneumophila* refers to all legionella bacteria, i.e. also those that are unable to reproduce on the selective agar medium.
Here, *Legionella* spp is a notation used in biology to designate all Legionella species.

Finally, the notation *Legionella (pneumophila)* is also used with some regularity in the report and this means that it has been demonstrated for *Legionella* spp, but also specifically for *L. pneumophila*.

2.2 Introduction regulatory framework

2.2.1 The regulatory framework consists of laws, Executive Orders, regulations and NEN standards

The rules for legionella prevention in drinking water and warm tap water systems can be found in the Drinking Water Act, the Housing Act, the Drinking Water Decree, the Building Decree 2012, the Drinking Water Regulations, the Regulation for legionella prevention in drinking water and warm tap water and the Regulation for materials and chemicals in drinking water and warm tap water supplies. Furthermore, NEN1006 (and its elaborations in the Water Worksheets) is very relevant because it is mandatory in
Both the Drinking Water Decree and the Building Act 2012. For the 2012 Building Decree the qualification that the equivalence provision in Section 1.3, subsection 1, of the 2012 Building Decree applies. This means that in theory it is possible to deviate from NEN 1006 if a different
solution 'offers at least the same degree of safety, health protection, usability, energy efficiency and environmental protection as is intended by' the regulations in NEN 1006. Furthermore, the regulatory framework also refers to some other standards.

In this study, we describe not only the content of the rules and whether they need to be adapted to scientific insights, but also where, i.e. in which part of the regulations, the rule in question is
included. This is important for two reasons. First, it makes it possible, in the follow-up phase of this study, to propose targeted regulatory adjustments.

Second, this insight is important because *where* a rule is included determines the procedure that must be followed to amend a rule. For example, rules in an Act (think of the Drinking Water Act) can only be amended after a relatively long procedure in which the government, the Council of State and the Lower and Upper Houses have a role. Compared to a law, it is easier to amend General Administrative Orders (AMvBs), such as the Drinking Water Decree and Building Decree 2012. These AMvBs are adopted by the government and submitted to the Council of State for advice.

The ministerial regulations (the Regulation on Legionella prevention in drinking water and hot tap water and the Regulation on materials and chemicals for drinking and hot tap water supply) are the
most easily adjusted. These are determined by the minister, who does not pass them on to other members of the government or the Council of State. Furthermore, the standards, such as NEN standards, are not set by the government, but by independent institutes. Adjusting these rules is therefore not a power of the government.

2.2.2 The distribution of rules among laws, Executive Orders, regulations and NEN standards

As follows from the previous paragraph, the rules on legionella prevention in drinking water and hot tap water systems are divided among laws, AMvBs, regulations and standards. Behind this division are a number of design principles, which we briefly discuss in this section.

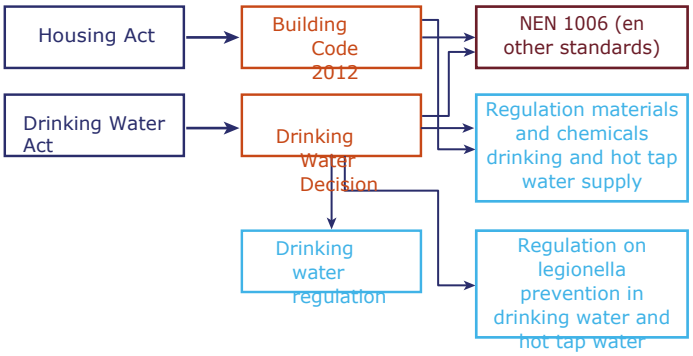


Figure 2.

First, powers that directly interfere with the freedom of citizens and companies must be regulated at the legislative level (i.e., in the Drinking Water Act). An example is the granting of powers to officials to apply administrative coercion (Article 50, paragraph 1, Drinking Water Act). With this power, the government can impose penalty payments on citizens and companies or even at a citizen's expense
or a company directly intervene in drinking water and heat tap water systems. These are far-reaching measures for which the government must be able to present a solid democratic legitimation. That is why rules of this kind are only made in a law, so that the Council of State can advise on them and the government and parliament have agreed to them.

At the same time, because the rules in laws require a time-consuming procedure, the legislature chooses not to include detailed and/or technical requirements in the law. Technical requirements also do not require the democratic legitimacy of a legislative procedure. Often, in these cases, the inclusion of these rules in a ministerial regulation is sufficient.

This is also readily apparent in the rules for legionella prevention. For example, the Regulation on the prevention of legionella in drinking water and domestic hot water consists largely of appendices in which 'Measurement frequencies in connection with the performance of measurements for the presence of legionella bacteria in the drinking water in the distribution area by a drinking water company' are elaborated, or the 'Regulations for the performance of a legionella risk analysis' are listed. Something similar can be seen in the Regulation on materials and chemicals for drinking and tap water supply.

Between the laws and the regulations are the General Administrative Measures (AMvBs). These are less detailed than regulations, but more detailed than laws. They are rules for which the light procedure of ministerial regulation offers too few guarantees, but for which the law is too heavy an instrument. An example is the target regulation in article 37, paragraph 1, of the Drinking Water Decree. This stipulates that an owner of a collective water supply must ensure that a legionella risk analysis is carried out. What this risk analysis should entail exactly are details that have been worked out in a ministerial regulation (in this case appendix 2 of the Regulation on the prevention of legionella in drinking water and warm tap water), while the authority to enforce this obligation is in the Drinking Water Act (article 50, paragraph 1).

In the regulatory framework, NEN 10063 occupies a prominent position. This standard has been made mandatory4 for buildings (within the meaning of Section 1, paragraph 1, of the Housing Act) in the articles
6.12 and 6.13, of the 2012 Building Decree, and for collective lei- ding networks (insofar as they are not part of a building) in article 34, paragraph 1, of the Drinking Water Decree. NEN 1006 consists mainly of technical regulations. The position of this standard is remarkable because NEN standards are established by an institute without legislative authority and therefore not by a government body. Moreover, these standards are only available after payment to an institute. This raised the question some years ago whether this way of referring is lawful. The administrative law department of the Council of State ruled in 2011 that this method of referencing is indeed possible in a lawful manner. This means that NEN 1006 has a legal force comparable to a rule drawn up by a government body.
a legislator has been drawn up. However, changing a NEN standard is usually far from simple: it often requires the agreement of parties with different interests.

3 Which version of NEN1006 applies is regulated in a ministerial regulation (see article 1, part b, of the Drinking Water Decree and articles 6.12, second paragraph, and 6.13, second paragraph, of the Building Decree 2012).
4 With the aforementioned nuance due to the application of the equivalence provision in article 1.3, paragraph 1, of the Building Act 2012.

2.2.3 The distinction between facilities in priority settings and facilities outside priority settings

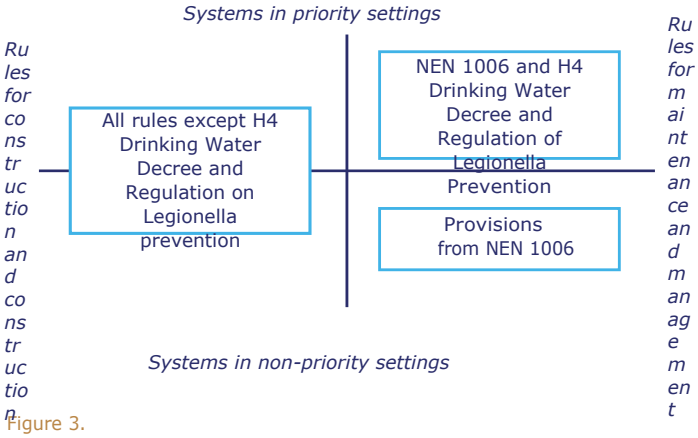


Figure 3.

Within the rules on legionella prevention in drinking water and hot tap water systems, the distinction between pipe networks and installations in priority settings and pipe networks and installations outside priority settings is relevant. Based on the 2012 Building Decree and Drinking Water Decree, NEN 1006 applies to all piping networks and installations.
The same applies to requirements for the materials from which pipes must be made, via the Regulation on materials and chemicals for drinking water and hot tap water supplies. NEN 1006 and the aforementioned regulation mainly set requirements for the *construction or installation* of piping systems and installations. NEN 1006 also contains minimum requirements for the management and maintenance of systems.

For priority establishments, on top of the minimum requirements, additional rules apply for the management and maintenance of piping networks and installations. These rules can be found in chapter 4 of the Drinking Water Decree and the Regulation on Legionella Prevention.
in drinking water and hot tap water. Article 35 of the Drinking Water Decree lists all the establishments that are priority establishments. The reason for setting additional rules for the priority establishments is because in these priority establishments the risk of vulnerable people becoming infected with *Legionella* is greater and therefore additional safeguards are necessary on top of a proper construction or construction of a piping network or installation.

When an establishment is a priority, the owner of a collective water supply or collective piping system is obliged to have a legionella risk analysis done by a certified company (article 37, of the Drinking Water Decree). If this analysis gives cause to do so, this also leads to an obligation
to have a legionella management plan drawn up by a certified company (Article 38, of the Drinking Water Decree).
This management plan an owner must be able to submit to an inspector (Article 39, of the Drinking Water Decree) and
(article 40 of the Drinking Water Decree). Furthermore, the owner has an obligation to report (article 41, of the Drinking Water Decree), there are requirements for the manner and frequency of sampling and analysis (articles 42 and 43, of the Drinking Water Decree) and the sequence of control measures is prescribed (articles 44, of the Drinking Water Decree). These provisions are further detailed in the Regulation on Legionella Prevention in Drinking Water and Hot Tap Water.

2.2.4 Related regulations

As described above, in this analysis we limit ourselves to rules on legionella prevention in drinking water and

hot water systems. However, *Legionella* is not limited to these systems. Also in other systems, where the water is between 20 and 55°C, *Legionella* can be present in sometimes very high concentrations. One can think of wet cooling towers, swimming pools, ships and airplanes. Rules for prevention of *Legionella* in these systems are contained in - or can be imposed on the basis of - the Working Conditions Act, the Environmental Management Act, the Public Health Act and the Swimming Facilities Hygiene and Safety Act, and the Municipalities Act. We do not include these rules in our analysis. It is, of course, possible that the scientific insights that emerge in this report are also relevant to these rules. Furthermore, it is relevant that some of the rules regarding legionella prevention land in the Environment Act and underlying regulations. This applies to the building regulations and the rules for swimming in water basins, wet cooling towers and wastewater treatment plants, among others.

The current legionella regulations do not have a European origin. It is only in the new Drinking Water Directive that regulations for legionella prevention in drinking water are included. It is
It is therefore important that any changes to national regulations fit within the European legal framework. An example is the mandatory analysis method (culture method) in the new Drinking Water Directive. Furthermore, the European
loyalty principle that Dutch governments take into account non-legally binding agreements made, for example, within the *European Center for Disease and Control*.

2.2.5 Definitions

The regulations contain some a number of definitions, which we use in this report.

Definitions of water

- *Drinking water means* water intended or partly intended for drinking, cooking or preparing food or for other domestic purposes, excluding hot tap water, which is made available to consumers or other customers through pipes;
- *Hot tap water:* water intended or partly intended to be drink, cook or prepare food or for other domestic purposes, which is heated before being made available for those uses.
- *Domestic water:* water intended exclusively for toilet flushing.

Definitions of type of water supply

- *Public drinking water supply:* production and distribution of drinking water by drinking water companies.
- *Collective water supply:*
  - land-based facility, other than a drinking water company, for the production or distribution of water that is made available to consumers or other customers as drinking water or hot tap water by means of a pipeline or distribution network
  - facility for the production or distribution of water at a mining installation located within Dutch territory (is a mining installation attached to the bottom of a surface water) which water is made available as drinking water or hot tap water to consumers within that mining installation.

Definitions of piping networks and installations

- *Collective piping network:* set of pipes, fittings and appliances which are temporarily, but not for the purpose of supply, or permanently, connected to the distribution network of a drinking water company or collective water supply, and through which drinking water or hot tap water is made available to consumers or other customers.
- *Dwelling installation:* part of a dwelling assembly of pipes, fittings and appliances connected to the piping network of a drinking water company or a collective water supply or to a collective piping network.
- *Tapping point:* place where drinking water, household water or Hot tap water becomes available for use.
- *Aerosol-forming tapping point:* aerosol-forming tapping points are defined as:
  - taps with a shower or other appendage that allows water to be sprayed or misted
  - taps used, temporarily or otherwise, to connect a shower, other appendage or device capable of spraying or fogging water
  - Taps that the owner reasonably knows or suspects will be used, temporarily or otherwise, to connect to a shower, other appendage or device capable of spraying or fogging water
  - all taps in an institution as referred to in subsection 1(a), to the extent that it is a department is hematology or oncology, or transplants are performed there or patients with chronic lung disease or disorders of the immune system reside there.

CHAPTER 3

Influence of hot water temperature on Legionella in drinking water systems of buildings

3.1 Current legislation

3.1.1 Residential Installations

For residential installations, many of which are small in size, no specific policy has been developed in terms of legionella prevention. Based on the 2012 Building Decree (which refers to NEN 1006), a requirement of 55°C applies to the mixing device or tap point for domestic hot water systems without a circulation system. However, that requirement was at the time
included primarily for a functional reason (specific domestic use for cleaning and dishwashing) and not as a Legionella control measure. Of course, in practice this requirement also has a preventive effect on the growth of Legionella.

3.1.2 Collective piping network

All collective pipe networks

The first paragraph of article 34 of the Drinking Water Decree stipulates for collective piping networks (of both priority and non-priority establishments) that the pipework must comply with NEN 1006, to the extent that they are not part of a building. As far as they are part of a building, NEN 1006 applies on the basis of
of the 2012 Building Decree (sections 6.12 and 6.13). For collective tap water systems, NEN 1006 stipulates that for tap water facilities and tap water systems with circulation, the temperature in the return pipe(s) must be at least 60°C when used in accordance with the design conditions.

This also applies to the temperature at the mixing device or at the tap point. Furthermore, on the basis of NEN 1006 Requirements for the temperature in relation to standing time. If a temperature of at least 60°C is not continuously maintained in all areas of a hot water storage tank, it must be thermally disinfected at least weekly in accordance with Table 4 in the standard to prevent bacteriological regrowth: at least 20 minutes at 60, 10 minutes at 65 and 5 minutes at 70°C.

Collective water supply and collective piping systems of priority institutions

For priority institutions, in addition to articles 6.12 and 6.13 of the Building Act and article 34 of the Drinking Water Decree also chapter 4 of the Drinking Water Decree and the elaboration of chapter 4 in the Regulation legionella prevention in drinking water and warm tap water. Annex 2 of this document contains regulations for performing a risk analysis and requirements for the risk factors to be used in the risk analysis and qualification of risk. A water temperature of between 25
and 50°C is considered a risk factor. Risk reducing factors are water temperatures below 20°C, between 20
and 25°C as far as there is a maximum of one week of downtime and good flow, water temperatures above 50°C, water temperatures above 60°C (in connection with killing legionella bacteria), flow-through and short residence time.

If favorable growth conditions occur and the owner chooses to control by post-heating the water or by increasing the temperature of the piping system weekly (thermal disinfection), the owner shall maintain one of the following ratios of temperature and time: for post-heating time 10 minutes at 60°C, 1 minute at 65°C or 10 seconds at 70°C and for the standby time with weekly preventive thermal disinfection: 20 minutes at 60°C, 10 minutes at 65°C or 5 minutes at 70°C.

Furthermore, Annex 2 of the Regulations contains a table (5.2) for risk classification based on temperature and duration in a component of the tap water system. This should be re-evaluated on the basis of the latest scientific insights. The '1 liter rule' (see Chapter 8) is also covered by this.

3.2 Reason to include topic in evaluation

In 2019, the report 'Possibilities for lowering the required temperature of hot tap water - research t.b.v..
Van der Lee motion (34 902)' appeared (Van Wolferen, 2019). That report describes a study of whether it is possible in homes to reduce the hot water temperature from 55°C.
The report drew the following conclusion about lowering the hot tap water temperature in relation to legionella risk:

'A reduction in the required temperature is possible without legionella risk, according to the Legionella Prevention Regulations, provided the following conditions are met:

- *Stock tanks and flow-through units that are continuously maintained at temperature shall be thermally disinfected at least weekly by increasing the temperature in the entire vessel or heat exchanger for a prescribed standby time.*
- *Flow-through cooling devices with a water content of the heat exchanger (drinking water side) smaller than one liter are allowed without further management measures.*
- *The pipe volume between the appliance and each individual tap point should be a maximum of 1 liter.*

A reduction in the required temperature requires that NEN 1006 specify the above conditions regarding legionella safety.'

The RIVM and KWR Water Research Institute sent a response to the report to the commissioners stating that scientific studies have shown that lowering the hot tap water temperature in combination with weekly thermal management results in an increased risk
on growth of Legionella. Thus, based on this response, the current Legionella Prevention Regulations and the aforementioned report (van Wolferen, 2019) do not appear to be in line with current scientific understanding.

3.3 Brief overview of scientific insights for 2001

Thermal management strategies can be divided into two categories: preventive and curative (National Academies of Sciences, 2019). By preventive, it is meant that prevented culturable *Legionella* spp from multiplying in the tap water system to a level above the legal standard (100 cfu/l) and curative means that if culturable *Legionella* spp has multiplied in the tap water system, a measure is taken that kills off the culturable Legionella bacteria to levels below the legal standard.

3.3.1 Maintaining high water temperatures

Prior to 2001, it was known from laboratory studies that at temperatures above 50°C, decay occurs of *L. pneumophila*. The decimal reduction time (this is the time needed to kill off 90% of the microorganisms) thereby decreases with increasing temperature. At 50°C, this decimal reduction time is 100 minutes for culturable *L. pneumophila* that had been reared under natural conditions (Van der Kooij, 2014), while at 60°C the decimal reduction time decreased to about 2 minutes (Dennis *et al*., 1984, Schulze-Robbecke *et al*., 1987, Van der Kooij, 2014). A decimal reduction time around 2 minutes was also observed at 70°C for culturable *L. pneumophila* raised as a pure culture (Stout *et al*., 1986).

The study by Stout *et al*. (1986) also showed that the decimal reduction time for the different temperatures tested varied between legionella species but also between strains of *L. pneumophila*, with the decimal reduction time of 2.6 minutes being observed for the most heat-resistant strain.

In addition to the aforementioned laboratory studies, studies have also been conducted on the influence of hot water temperature on the presence of culturable *Legionellaspp* or *L. pneumophila* in drinking water systems of different buildings. The scientific studies published on this subject before 2001 have shown that *L. pneumophila* can be cultured more frequently from the tap water system when the temperature of the hot water from the hot water preparation system is below 60°C than when the temperature of the hot water from the hot water preparation system is 60°C or higher (e.g. Plouffe *et al*., 1983, Arnow *et al.* , 1985, Groothuis *et al.* ,
1985). Those scientific insights have been an important reason for thermal management to be leading in Dutch legislation to control culturable *Legionellaspp* in drinking water systems.

3.3.2 Thermal disinfection

Prior to 2001, hardly any scientific studies were available on the effects of (periodically) temporarily increasing the hot water temperature, called heat shock, on the numbers of *Legionella* in the tap water system.

3.4 Overview of scientific insights since 2001

3.4.1 Maintaining high water temperature

Decimal reduction time

Because *L. pneumophila* reproduces in host protozoa in tap water systems, there have also been recent studies on the influence of thermal management on the decimal reduction time of host protozoa *Acanthamoeba* spp and *Vermamoeba vermiformis* (Cervero-Arago *et al.* , 2013). The results of that study showed that at 50 and 60°C, the decimal reduction time of particularly the cyst form of *Acanthamoeba* (76 minutes at 50°C and 10.7 minutes at 60°C) and *V. vermiformis* (30 minutes at 50°C and 4.7 minutes at 60°C) are longer than those of culturable *L. pneumophila*. This implies that the decimal reduction time of culturable *L. pneumophila* in a cyst of *Acanthamoeba* and/or *V. vermiformis* is longer at a given warm water temperature than for culturable planktonic *L. pneumophila* cells
in the water. A 2004 study confirms this picture, as it observed that 60 minutes of incubation at 60°C resulted in ~6 log reduction of culturable planktonic
L. pneumophila cells, but only a ~4 log reduction of culturable *L. pneumophila* cells in cysts of *Acanthamoeba* (Storey *et al.* , 2004).

Influence of high water temperature on Legionella *in drinking water systems*

A large number of studies on the effects of high drinking water temperatures on *Legionella (pneumophila)* have been conducted under controlled conditions in the laboratory.
However, potable water systems used in buildings do not operate under these controlled laboratory conditions. For example, the water temperature achieved by the water heater in a building is not equivalent to
the water temperature reached at the faucet. A controlled study showed that hot water at the faucet could cool to room temperature (24-25°C) within 30 minutes, while water from the water heater had a temperature of 58°C (Rhoads *et al.* , 2015). In doing so, it was incidentally observed that when convection mixing occurs in the pipe, the water temperature does not cool further than 39°C, which is an ideal temperature for growth of *L. pneumophila*.

The issue of convection mixing is discussed further in Section 9.4.3. Large buildings, such as hospitals for example, therefore have loop pipes where hot water is in recirculates to the water heater. This prevents extensive cooling of hot water in the hot water system, although in buildings with such loops it has also been observed that at some distal points of the piped water system the water temperature can fluctuate widely, also reaching temperatures (30 - 40°C) that give an increased risk of *Legionella* growth, while the average water temperature after the boiler was above 60°C (Bedard *et al.* , 2015). This study also showed that in addition to hot water temperature, frequency of water use was an important parameter in the extent to which hot water temperature at a distal point cooled to temperatures in which *L. pneumophila* is able to reproduce.

Several studies have been published in the scientific literature where the influence of hot water temperature was investigated on the presence of culturable *Legionella spp* or *L. pneumophila* in drinking water systems of different buildings. After 2001, several scientific studies have been published that have confirmed that *L. pneumophila* and or other culturable Legionella species can be well controlled at a hot water temperature of 60°C or higher from the hot water preparation system and/or a
hot water temperatures of 55°C or higher at the taps, but that at lower temperatures (including at temperatures between 50 and 55°C) tap water systems become more likely to be positive for *L. pneumophila* and/or other cultivable legionella species (Darelid *et al.* , 2002, Blanc *et al.* , 2005, Borella *et al.* , 2005, Saby

et al. , 2005, Mouchtouri *et al.* , 2007, Hrubá, 2009, Arvand *et al.* , 2011, Barna *et al.* , 2016, Bedard *et al.* , 2016, Boppe *et al.* , 2016, Lecointe *et al.* , 2019). These studies and those prior to 2001 were conducted in different countries (and thus with different water quality), on drinking water with or without disinfection residue, and in different building types (with or without a loop). The general finding is that, regardless of water quality, presence of disinfection residue and building type, culturable *Legionella (pneumophila)* remains controllable at a hot water temperature of 60°C or higher of water from the water heater combined with a hot water temperature of 55°C or higher at the tap.
This shows that such thermal management is successful in preventing the risk of growth of culturable *Legionella* (pnemophila) in the tap water system. This picture has also been confirmed, in which through a modeling approach, data from eleven independent field studies were used to derive the threshold value for thermal management (Rasheduzzaman *et al.* , 2020). The results showed that according to the statistical methodology (odds ratio or regression) 55°C or 59°C is an appropriate threshold for hot water temperature at taps.

The last few decades have also shown that not all *L. pneumophila bacteria* are culturable with the selective agar medium and that with the use of host protozoa, for example, samples are found positive for
L. pneumophila that were negative with culture on an agar medium (Schalk *et al.* , 2012). In many cases, the negative culture could also have been caused by the overgrowth of interfering flora on the agar plates, which may have allowed *Legionella* to grow on the medium, but because the interfering flora is present in higher numbers, was not detected. Bacterial cells that do not grow on an agar plate, but are detected by other methods that demonstrate cell viability, are also called viable but non-culturable (VBNC) cells. Several methods are used in this regard and commonly used methods include DNA from membrane-intact *L. pneumophila* (qPCR with ema or pma) or detection of membrane-compromised cells versus membrane-intact cells (Delgado-Viscogliosi *et al.* , 2009, Cervero-Aragó *et al.* , 2019). However, there is debate among scientists whether each of these methods can reliably detect VBNC cells of *L. pneumophila* (National Academies of Sciences, 2019). To determine the influence of high water temperature (55, 60 and 70°C) on culturable and VBNC *L. pneumophila*, high numbers (1×108 culturable cells/ml) of two *L. pneumophila* strains were exposed to one of these three different water temperatures for 80 days (Cervero-Aragó *et al.* , 2019).

The results showed that an 8 log removal of culture-able *L. pneumophila* was observed after 3 to 8 hours at 55°C, 60 minutes at 60°C and less than 2 minutes at 70°C. However, the VBNC cells of *L. pneumophila*, based on an intact cell membrane and esterase activity, were more persistent and a 4 log removal of the VBNC cells was observed after 150 days at 55°C, 8 to 15 days at 60°C and 1 to 4 days at 70°C. However, the infectious status of these VBNC cells was also investigated using a host protozoa and lung ma- crophage cell line and showed that infectious *L. pneumophila* cells were still observed up to 85 days after incubation at 55 to 60°C and up to 8 days at 70°C. In these analyses, however, it remains unclear whether propagation of *L. pneumophila* occurred from VBNC cells or from residual culturable *L. pneumophila* cells that were not detected because the numbers were below the detection limit of the culture method. Cervero-Aragó *et al.* (2019) concluded from their results that a prolonged (> week) thermal regime of 60°C or higher in the central part of the hot water system should also be effective against infectious VBNC cells of *L. pneumophila*. However, such prolonged high temperatures are only achieved in the hot water supply of the hot water system.

In addition to these studies under controlled laboratory conditions, field studies have shown that when hot water temperatures of water from the water heater is 60°C or above and/or the hot water temperature at the taps of the tap water system is 55°C or above, not only do culturable Legionella numbers remain manageable, but also the number of VBNC or dead cells of *L. pneumophila* (determined by quanti- tative PCR (qPCR))(Bedard *et al.* , 2015, Lecointe *et al.* , 2019).

In a recent report by the National Academy of Sciences, Engineering and Medicine, a committee of legionella experts concluded the following regarding preventive thermal management, based on current scientific understanding:
'Several studies
have demonstrated, at the level of different scales, countries and building types, the overarching benefit of elevated temperatures to control *Legionella*.
In particular, boiler settings above 60°C are a key threshold value to reduce the number of positive detection cases of *Legionella* as well as to reduce the number of Legionnaires' disease cases and Legionella outbreaks. Adjusting the boiler outgoing water temperature to a value that provides drinking water temperatures higher than 55°C at distal taps can be very effective in reducing the number of Legionella-positive swabs or water samples
reduce."(National Academies of Sciences, 2019).

Finally, it is important to note that thermal management with a continuous high hot water temperature is effective in the hot tap water lines up to the thermostatic mixer. *Legionella,* which may be present in the pipe and/or shower head after the thermostatic mixer tap, is not affected by thermal management. However, the part after the thermostatic mixing valve of a tap water system has a volume less than one liter, of which the current regulations indicate that such parts have a neutral risk for Legionella. Chapter 8 discusses this so-called one-liter rule.

3.4.2 Thermal disinfection

Since 2001, several scientific studies have been published investigating the effect of heat shock on *Legionella*. In the different studies, these heat shocks have been applied in different forms, that is, different heat shock temperatures, duration of heat shock and frequency of heat shock. In addition, the study was conducted in different systems, viz. under laboratory conditions, in pilotscale drinking water systems using modeling and in buildings under field conditions. In the following, the results of scientific studies obtained under laboratory conditions or in pilotscale tap water systems are described first, then the results of a modeling study, and finally the results of scientific studies obtained in buildings.

Laboratory and pilot scale studies

In the laboratory and pilot scale studies found on the in- vloed of heat shock on *Legionella* and/or *L. pneumophila*, the heat shock temperature, time of heat shock, and frequency of heat shock administration varied (Table 1).

Table 1. **The heat shock protocols applied in the laboratory and pilot scale studies.**

Heat ShockTemp					
Temp	***Duration***	***Frequency per experiment***	***hot water***	***Installation***	***Reference***
70°C	30 min	Vaaka	37°C	Pilot DW	(van der Kooij *et al.* , 2005)
70°C	30 min	2	35°C	Pilot DW	(Farhat *et al.* , 2010)
70°C	30 min	2	35°C	Pilot DW	(Farhat *et al.* , 2012)
70°C	0 60 min	1	N.P. [b]	Laboratory	(Allegra *et al.* , 2008)
50 70°C	5 60 min	1	N.P.	Laboratory	(Epalle *et al.* , 2015)
70°C	30 min	1	40°C	Pilot DW	(Saby *et al.* , 2005)
65°C	30 60 min	4	45°C	Pilot DW	(van Kenhove, 2018)
60°C; 65°C	30 min	>10	45°C	Pilot DW	(Bleys & Dinne, 2020)
60°C	30 min	1	40°C	Pilot DW	(Ji *et al.* , 2018)

aInthis study, flushing was done twice a week for a period of approximately 85 weeks
bN.P.has not been published

Usually a heat shock regime of 30 minutes at 70°C was applied The most intensive heat shock examined in these studies was 60 minutes at 70°C, while the least intensive heat shock was 30 minutes at 60°C or 5 minutes at 70°C.
The frequency of heat shock administration varied between a frequency of once to a frequency of twice a week for approximately 85 weeks. In addition, all studies applied a relatively low hot water temperature after heat shock, so the studies were particularly
provide information on control of *Legionella (pneumophila)* when would be allowed to lower the hot water temperature. The general picture that emerges from the laboratory and pilot scale studies is that heat shock results in a temporary reduction in the number of *Legionella (pneumophila)*, but that often not all *Legionella (pneumophila)* could be killed off and that after some time the numbers are back to the old level or even higher than before
The heat shock (Saby *et al.* , 2005, van der Kooij *et al.* , 2005, Allegra *et al.* , 2008, Farhat *et al.* , 2010, Epalle *et al.* , 2015, Kruse *et al.* , 2016, van Kenhove, 2018, Bleys & Dinne, 2020). In this regard, the study by der Kooij *et al.* (2005) was conducted with Dutch drinking water in a pilot tap water system and used a frequency (twice a week) most similar to that used in Dutch practice (weekly heat shock). It was also observed that the biofilm is hardly removed by the heat shock and that host protozoa of *Legionella* are also present after applying a heat shock treatment (Farhat *et al.* , 2012) in a pilot plant. In addition, it follows from these studies that the application of thermal disinfection in combination with a reduced hot water temperature leads to conditions in which *Legionella (pneumophila)* can maintain or even multiply to higher numbers than when no heat shock is applied.

In one of the studies, it was observed that the numbers of culturable *L. pneumophila* bacteria decreased after a first heat shock, but that a second heat shock, applied when the numbers of *L. pneumophila were* back to the old level before the first heat shock, did not result in a decrease but in a temporary increase in the number of culturable *L. pneumophila (*Farhat *et al.* , 2010). These results show that *L. pneumophila* can become hitteresistant when repeated heat shocks are applied. In addition, the study also shows a second risk of applying heat shock, which is that the numbers of culturable *Legionella* can increase after applying heat shock. A similar result was obtained in a pilot distribution system where after applying a heat shock (30 minutes at 70°C) the numbers of *L. pneumophila* in the biofilm initially decreased by more than 3 logen units. However, after the water temperature in the water system was returned to 40°C, the culturable legionella numbers in the biofilm increased again and these numbers were 2 logen units higher after five weeks than those observed before the heat shock (Saby *et al.* , 2005). Temmerman *et al.* (2006) showed that culturable numbers of *L. pneumophila* increased when bacteria and biofilms that had been subjected to heat shock (30 minutes at 60°C) killed and were added to *L. pneumophila*. The authors concluded that *L. pneumophila* is able to reproduce directly on the nutrients released from culled microorganisms (a phenomenon called necrotrophy), i.e. without reproducing in a host protozoa. These results thus show that when heat shock is applied as a preventive measure there is a risk that hitteresistant *L. pneumophila* will predominate in the tap water system on which the heat shock has little effect and that these hitteresistant *L. pneumophila* strains can grow to higher numbers after a heat shock by multiplying on the dead biomass created in the system by the heat shock. Thus, with this, applying a heat shock strategy as a preventive measure could backfire and result in a higher risk of spreading culturable *Legionella* and *L. pneumophila*.

Modeling Research

A recent study used modeling to investigate whether a constant high hot water temperature (55°C) or a heat shock procedure is effective in controlling culturable *Legionella* in apartment buildings where relatively high culturable Legionella numbers are found (van Kenhove, 2018). The model simulations showed that growth of culturable *Legionella* was completely absent is with a constant high hot tap water temperature and that with a heat shock protocol, growth of culturable *Legionella* does occur between two administered heat shocks, but in doing so the culturable Legionella numbers remain below 1000 cfu/l. In the model, however, a number of made assumptions that do not match the knowledge about growth of Legionella. For example, growth of *Legionella* has been modeled as growth outside of host protozoa, whereas in drinking water biofilms, *Legionella* actually reproduces in host protozoa (National Academies of Sciences, 2019). Upon die-off by temperature is also assumed to be free-living Legionella cells, whereas it is known that this die-off is lower when *Legionella* is in host protozoa (Storey *et al.* , 2004; Cervero-Arago *et al.* , 2013). In addition, the growth of *Legionella* on dead biomass, which occurs after thermal shock treatment, has not been included, nor the evolution towards more thermotolerant Legionella species after heat shock treatment. Both phenomena are however, described in the scientific literature (Temmerman *et al.* , 2006; Allegra *et al.* , 2011). The consequence of these assumptions is that the growth of *Legionella* between two heat shock treatment is likely to be underestimated, while the shedding of *Legionella* by heat shock treatment is overestimated. As a result, the effect of heat shock treatment may appear more positive by these model simulations than it will be in reality. Further development of the model, including validation to standardized standards, may possibly lead to more reliable results in the future to predict the effect of heat shock treatments on *Legionella (pneumophila)*.

Practice conditions

The application of thermal disinfection was also investigated under practical conditions mainly in hospitals (Table 2). In some studies thermal disinfection was applied as a preventive measure, whereby the water temperature was temporarily increased over a long period of time, while in other studies thermal disinfection was applied as a curative measure, whereby the temperature was temporarily increased once or several times a year. The general picture that emerges from these studies is that in none of the published studies applying thermal disinfection led to the successful reduction of culturable *Legionella* spp at all sites to below < 100 cfu/l (Perola *et al.* , 2005; Peiro Callizo *et al.* , 2005; Bedard *et al.* , 2016; Allegra *et al.* , 2011; Kruse *et al.* , 2016; Mouchtouri *et al.* , 2007; Steinert *et al.* , 1998; Borella *et al.* , 2016; Marchesi *et al.* , 2011; Pancer *et al.* , 2013).

For example, in one of the studies where thermal disinfection was used preventively and the water was treated weekly with heat shock, it was observed that this strategy successfully reduced the number of culturable *L. pneumophila* to below 100 cfu/l in the facility of one of the two wings of the hospital (Bedard *et al.* , 2016).
However, application of the same thermal disinfection protocol to the installation of the other wing of the hospital was not successful. Varying results were obtained at the different taps of this installation. For example, at a couple of taps it was observed that during the first 12 months of applying the thermal disinfection protocol the culturable numbers of *L. pneumophila* remained unchanged high (^{104}-105 cfu/l), while at another tap point these numbers decreased to ~ ^{102} cfu/l.
At a fourth tapping point, it was observed that for the first six months after thermal disinfection was initiated, the numbers of culturable *L. pneumophila* dropped below 100 cfu/l, but after 12 months the numbers had increased substantially (5 x105 cfu/l). The authors suggest
That the difference in success of thermal disinfection between the two plants is caused by the difference in hydraulic conditions between the plants. Indeed, in the plant where thermal disinfection was not successful, many more dead-end pipes occurred than in the
plant where thermal disinfection was successful. In the study by Peiro Callizo *et al.* (2005), it was also observed that thermal disinfection was successful in reducing culturable *Legionella* below the detection limit (50 cfu/l) in one part of the plant, but not in the other part. In
the unsuccessful part, legionella numbers were reduced, but remained relatively high at 1,950 cfu/l. In addition, it is difficult to assess whether the decrease in culturable legionella numbers was caused by thermal disinfection, because at the same time dead-end pipes were also removed and thermal management was improved (temperature from hot water heater was brought to 60°C or higher and return pipe temperature at least 50°C, before this these temperatures were apparently lower). Finally, it was observed in this study that sanitizing a dead-end pipe in the plant where thermal disinfection was unsuccessful resulted in further reduction of the number of culturable *Legionella* to below the detection limit (50 cfu/l). This is another indication that the success of thermal disinfection as a preventive measure also depends on the hydraulics of the plant.

The third study where thermal disinfection was used as a preventive measure did not see a positive effect on the numbers of culturable *L. pneumophila* and the numbers therefore remained unabated in the system (up to a maximum of ^{105} cfu/l). In this last study, the numbers found were *L. pneumophila* also typed to strain level (genotype) and those results showed that prior to application of the thermal disinfection protocol, three different legionella strains were observed, but then two of the three strains remained. This potentially shows that thermal disinfection ultimately selects for *L. pneumophila strains* that are heat resistant, as has been demonstrated in the laboratory and pilot scale studies described previously.

The studies that have used heat shock as a curative measure under field conditions show that in almost all studies a reduction in culturable *Legionella (pneumophila)* was observed after heat shock (Allegra *et al.* , 2011; Kruse *et al.* , 2016; Mouchtouri *et al.* , 2007; Steinert *et al.* , 1998;
Borella *et al.* , 2016). However, in most cases this effect was temporary and culturable Legionella numbers increased again over time, sometimes to numbers higher than before heat shock. The time between heat shock and regrowth of culturable *Legionella (pneumophila)* varied among the various studies. In some studies, regrowth to similar or higher numbers was observed within 7 to 14 days (Steinert *et al.* , 1998; Pancer *et al.* , 2013), but in other studies only
after 60 days (Borella *et al.* , 2016). Another interesting observation from these studies is that applying heat shock was more effective for *L. anisa* or L. nonpneumophila than for *L. pneumophila (*Kruse *et al.* , 2016; Mouchtouri *et al.* , 2007). For example, it was observed that when
L. anisa and *L. pneumophila* were present in the system before heat shock, *L. anisa* was no longer detected after heat shock, but *L.* pneumophila was (Kruse *et al.* , 2016).
Also, another study showed that the heat shock led to a shift within the *L. pneumophila population*, with only hitteresistant strains of L. pneumophila found after the heat shock, because some were different strains than those found before the heat shock.

Table 2. **The heat shock protocols applied in the field studies and result regarding legionella control.**

Temp	Duration	Strategy	Frequency	Installation	Good luck	Reference
80°C	5 min	Preventive	N.P.	Hospital	No	*Perola et al., 2005*
6670°C	> 3 min	Preventive	12 per week	Hospital	Mixed	*Peiro Callizo et al., 2005*
> 70°C	≥ 30 min	Preventive	Weekly	Hospital	Mixed	*Bedard et al., 2016*
70°C	30 min	Curative	12 per year	Hospital	Temporary	*Allegra et al., 2011*
65°C	≥ 24 hours	Curative	15 per year	Hospital	Temporary	*Allegra et al., 2011*
65°C	≥ 24 hours	Curative	15 per year	Hospital	Temporary	*Allegra et al., 2011*
N.P.			N.P. per N.P.	77 buildings	Mixed	*Kruse et al., 2016*
7080°C	3 days	Curative	1 per 9 to 14 days	33 buildings	Mixed	*Mouchtouri et al., 2007*
70°C		N.P. Curative	1 per 98 days	Hospital	Temporary	*Steinert et al., 1998*
6065°C		N.P. Curative	1 per year	Hospital	Temporary	*Borella et al., 2016*
> 60°C	2 days	Curative	2 per year	Hospital	No	*Marchesi et al., 2011*
7080°C		N.P. Curative	12 per year	Hospital	No	*Pancer et al., 2013*

Studies investigating whether more intensive heat shocks (for example, several hours at 70°C or shorter times at temperatures of 80 or 90°C) are able to control *Legionella* in drinking water systems have not been found. Additional research is therefore needed before it can be concluded whether more intensive heat shocks do work as a preventive measure for *Legionella*. In doing so
It should also be noted that such more intense heat shocks can also cause potential problems. such as (i) the loosening of particles from the pipes that can lead to clogging of valves and strainers in the tap water system, (ii) the occurrence of damage to (parts of) the tap water system due to (long-term) exposure to high temperatures, (iii) scale formation in the plant and (iv) incurring burns by users of the hot water system (National Academies of Sciences, 2019).

Due to the mixed results in scientific publications regarding thermal disinfection on *Legionella* (pneumophila), it has been concluded that the effectiveness of the heat shock measure against *Legionella* in drinking water systems is
is controversial (National Academies of Sciences, 2019).
Therefore, in practice, a heat shock strategy is mainly used as a temporary or emergency measure, but not as a preventive measure (National Academies of Sciences, 2019).
In three scientific studies found, the strategy of a constant high hot water temperature (60°C) was compared to the heat shock strategy on controlling
L. pneumophila in pilot and fullscale tap water systems (Allegra *et al.* , 2011, Ji *et al.* , 2018, Bleys & Dinne, 2020). In these studies, it was observed that *L. pneumophila* was controlled in the hot water system with a
constant high hot water temperature of 60°C or more, but that in the hot water system with a temperature of 40, 45 or 55°C where heat shocks of 30 minutes at 60, 65 or 70°C were applied, *L. pneumophila* was found in the water. Thereby, the results of Bleys and Dinne (2020) are easier to interpret because in this study the pilot plant was also inoculated with *L. pneumophila*.

The study by Bleys and Dinne (2020) is also important in relation to the possible reduction of hot water temperature trip in combination with thermal disinfection. Indeed, in that study, the hot water temperature of the pilot plant was reduced to 45°C and thermal disinfection was applied weekly as a preventive measure (weekly heat shock of at least 60°C for 30 minutes). In that study, it was observed that a heat shock of 30 minutes at 65°C had a greater effect on culturable *Legionella* than a heat shock of 30 minutes at 60°C,
but in both regimes the Legionella numbers in the system were not reduced below the detection level of 10 colony forming units (cfu) per liter and the culturable Legionella numbers were also above 100 cfu/l with some regularity. It was also seen that when the regular administration of heat shocks (~ 1 time per week) was interrupted for several weeks, the numbers of culturable *Legionella* increased again strongly to numbers of ^{106} - ^{107} cfu per liter. From this it can be concluded that lowering
of hot water temperature in combination with thermal disinfection is not a reliable control measure against culturable *Legionella.*

Dutch situation

The Regulation on Legionella Prevention includes preventive application of thermal disinfection as a control measure. Therefore, the most relevant studies for the Dutch situation are those in which the effect of preventive thermal disinfection was studied. As the previous description shows, the results of these studies are not unequivocal,
because successful results were obtained in some facilities, but not in others. In addition to these previously described studies, a fairly intensive case-control study is also performed in the Netherlands when legionellapneumonia is found in a patient, the so-called Source Detection Unit legionellapneumonia (BEL) study (den Boer *et al.* , 2015, den Boer *et al.* , 2016). However, the BEL examination is not performed to such detail
That it is known whether culturable *Legionella* have been found in facilities where heat shock treatments are used.

However, the BEL study did show that culturable *Legionella (pneumophila)* are more frequently observed in tap water systems of priority institutions such as hospitals
and hotels than in homes (den Boer *et al.* , 2016), which could be indirect evidence that heat shock treatments used primarily in priority settings can lead to growth of *Legionella (pneumophila)*. There
are, however, other factors (e.g., hydraulics, downtime, size of plant, etc.) that can cause differences between a piped water system of priority buildings and homes and that can also be responsible for the differences in culturable *Legionella (pneumophila)* found.

3.5 Conclusion scientific state of the art thermal management

Based on current scientific understanding, it is concluded that applying a continuous high hot water temperature of 60°C of the outgoing water from the hot water heater and of 55°C at all outlets through the hot water system is a reliable preventive management measure to control *Legionella* and *L. pneumophila*.

The application of heat shocks in a hot water system where the hot water has a temperature lower than 60°C is not recommended as a preventive control measure because scientific studies have shown that (1) *Legionella* and *L. pneumophila* are not sufficiently killed in the hot water system and (2) such measures can lead to (temporarily) increased numbers in the hot water system.

The effectiveness of controlling *Legionella* by applying heat shocks in a hot water system where the hot water has a temperature of 60°C or higher appears to vary between hot water systems. It may therefore be a successful control measure for one site, but less or unsuccessful for another site.

However, a heat shock treatment can be used as a curative measure when in a hot water system
has been observed that the numbers of culturable *Legionella* or *L. pneumophila* exceed the legal requirement. By a curative measure is meant here that an immediate risk to public health, due to high numbers of culturable *Legionella (pneumophila)* in the installation, can be reduced by a thermal shock treatment. Indeed, such a measure results in an immediate (but usually temporary) reduction in the number of Legionella bacteria, preventing the instantaneous risk of *Legionella* spreading. However, in this case, after applying heat shock as a curative measure, additional preventive measures must be taken that will ensure that culturable *Legionella* does not increase again in the hot water system after application of the heat shock.

3.6 Knowledge and experiences from practice

The controllability and manageability of the hot water temperature, particularly in collective tap water systems with circulation, is very difficult in practice. Boiler systems and thermostatic valves need a considerable bandwidth in order to regulate the DHW temperature to 55 or 60°C, for example. Several respondents therefore argue that legal rules related to the use of circulating water systems should be amended.
to the DHW temperature to include sufficient margin in connection with the practicability of those rules. The return temperature at the boiler and the return temperature in the sub-ring are the most important key thresholds for this.
values. The circulation system should be considered an extension of the storage tank, for which a requirement of 60°C then applies everywhere (in collective tap water systems).

The sanitation industry endorses the importance of thermal management and maintaining 60°C as the key threshold value for collective hot water systems. The positive effect of strictly maintaining that temperature is also demonstrated by practical experience. They argue for restraint in lowering tap water temperatures, for example, as a result of the energy transition. In care for the disabled, many smaller care units (care homes) are used and *Legionella* in hot tap water is well controlled there. keep by strictly maintaining a temperature of 60°C. Most problems therefore manifest themselves in the cold water. A respondent confirmed that more and more sampling in practice is focused on cold water taps and not on hot water taps.

Referring to the 2018 Kenhove study, one of the respondents said that it is necessary to revise the table temperature - stand time as included in Annex 2 of the Regulation. This should then result in longer stand times and/or higher temperatures at post-heating and/or weekly thermal disinfection.

Because mixing devices are often well concealed in practice (for example, above suspended ceilings), it is doubtful whether the part behind a mixing device will be included with thermal disinfection. Several respondents indicated that this is necessary since an important part of the legionella risk is behind the mixing device. One respondent experienced that around
75% of standard violations are found at
Shower mixers related to the shower.

In addition to the pipe section behind a mixing device, the fact that hot water discharge pipes do not cool sufficiently to below 25°C is also considered a risk. In such a situation, the effectiveness of a thermal shock treatment is limited. In practice, the success of a heat shock treatment is very situation dependent and is mainly determined by the complexity of the installation and the materials used. Several respondents also pointed out the risk of combi heat pumps in homes equipped with
A thermal disinfection program using an electric heater. The weekly thermal disinfection of these heaters focuses only on the storage tank and not on the rest of the installation.
storage tank is disinfected. Performing proper thermal disinfection in practice is no easy task, because it is always questionable whether all positions in the tap water system are sufficiently reached; think especially of the bottom of storage vessels for hot tap water. One respondent
notes that in practice homeowners - from an energy point of view - are increasingly questioning the need for the electric heater for thermal disinfection in combination with a (ground) heat pump, especially if the kitchen has a close-in boiler with which hot water can be drawn for cleaning purposes, among other things.

3.7 Recommendation to adjust regulations based on scientific insights

The latest state of scientific knowledge, as described in Sections 3.4 and 3.5, is not in line with current legislation on legionella prevention. As stated in paragraph
3.1 described, legislation on legionella prevention can cover residential installations, collective piping systems and collective piping systems of priority buildings. This is emphatically important for the hot tap water temperature, because for dwelling installations and collective piping systems of non-priority establishments, the hot tap water temperature is the only legionella control measure applied.

3.7.1 Residential Installations

For hot water for hot water systems without a circulation system in homes, a requirement of 55°C applies under the 2012 Building Decree. This requirement is primarily for functional reasons, which is why a recently published report it is stated that Legionella prevention by law does not prevent lowering the domestic hot water temperature. However, the scientific knowledge concerning *Legionella* shows that lowering the hot tap water temperature in domestic installations will lead to an increased risk of *Legionella* growth and may therefore pose a public health risk. The interviews showed that also in daily practice with Legionella prevention in the Netherlands the experience is that when the hot tap water temperature is set to 60°C or higher at the hot water unit, or higher than 55°C at the outlet points, samples are almost never positive for culturable Legionella. It therefore makes sense to bring the legislation more in line with current scientific knowledge and practical experience.

The advice based on scientific findings is to amend the legislation so that hot water systems without circulation systems in homes must meet the requirements that the hot water temperature in the hot water heater (storage and instantaneous water heaters) is 60°C at all locations and that the hot water temperature at the outlet points is 55°C. In addition, it should be explicitly stated that this must be met in the context of legionella prevention.

An adjustment in line with the above will affect the construction and installation of domestic hot water systems, as well as their use. The homeowners, manufacturers of sustainable heating sources/hot water appliances, installers and NEN standards subcommittee NEN 1006 are the main stakeholders in this process.

3.7.2 Collective piping networks regardless of priority or non-priority settings

Based on the 2012 Building Decree and the Drinking Water Decree, collective piping networks must comply with NEN 1006.
NEN 1006 states that the temperature in the return pipe must be at least 60°C when used in accordance with the design conditions, as well as the temperature at the mixing device or at the tap point. In addition, it is stated that if a hot water storage device does not maintain a temperature of at least 60°C continuously at all points, it must be disinfected at least weekly (see also section 3.1.2). The analysis of the scientific literature and practical experience has shown that a tap water temperature of 60°C at the return pipe of the tap water installation, the mixing unit and the tap point is a reliable preventive measure for keeping *Legionella (pneumophila)* under control. The same applies to hot tap water installations where post-heating is applied, provided it meets the standards set in NEN 1006 (post-heating time 10 minutes at 60°C, 1 minute at 65°C or 10 seconds at 70°C). The effectiveness of a weekly heat shock treatment with the given temperatures and standing times in Table 4 of NEN 1006 is, according to current scientific knowledge and practical experience, not a reliable preventive measure to control *Legionella (pneumophila)* if the hot water temperature is below 60°C is and may even result in increased Legionella numbers after heat shock treatment due to growth of *Legionella (pneumophila)* on the dead biomass (released after heat shock treatment).

The recommendation based on the scientific evidence is to maintain the following requirements for collective piping networks (of priority and non-priority institutions):

1. In the case of circulating hot water facilities and hot water systems, the temperature in the return line(s) shall be at least 60°C when operated in accordance with design conditions, and
2. That this also applies to the temperature at the mixing device or at the aerosol-forming tap point.
3. If favorable growth conditions occur and the owner elects to control by post-heating the water, the owner shall apply one of the following ratios of temperature and time for such post-heating: 10 minutes at 60°C, 1 minute at 65°C, or 10 seconds at 70°C.

In addition, it should be explicitly stated that this must be complied with in the context of legionella prevention. Furthermore, a requirement should be added that in a hot water storage device a continuous hot water temperature of 60°C is also achieved in all places. The it is obvious that the passages in Table 4 of NEN 1006 about thermal disinfection at certain temperatures and standing times should be deleted.

An adjustment in line with the above will affect the construction and installation of collective piping networks, as well as their use. The building managers, installation managers, installers and NEN norms subcommittee NEN 1006 are the main stakeholders in this process.

3.7.3 Collective piping networks priority institutions

For collective piping networks of priority institutions, in addition to the 2012 Building Decree and the aforementioned article 34 of the Drinking Water Decree, the regulations described in chapter 4 of the Drinking Water Decree and the elaboration of this in the Regulation for the prevention of Legionella in drinking water and warm tap water. In these regulations, an unlimited hot tap water temperature of 50°C is considered a neutral risk, and at hot tap water temperatures of at least one hour and higher than 55°C, rejection applies.

In line with what is described in section 3.7.2, current scientific knowledge and practical experience shows that a continuous hot water temperature of 60°C at the return line of the hot water system, the mixing unit and the tap point is a reliable preventive measure to control *Legionella (pneumophila)*. This means that, according to current scientific knowledge, a neutral risk or die-off can be said to exist when the hot tap water temperature at the taps is 60°C or higher. The regulations also offer the possibility of controlling culturable *Legionella*, if favorable growth conditions occur, by post-heating the water or by increasing the temperature of the piping network weekly (thermal management with heat shock treatments). Post-heating of the water is also a reliable preventive measure, provided it meets the standards set out in Appendix 2 of the Legionella Prevention Regulations and NEN 1006 (post-heating time 10 minutes at 60°C, 1 minute at 65°C or 10 seconds at 70°C). A weekly heat shock treatment with the given temperatures and standing times in line with the Regulations on Legionella Prevention in Drinking Water and Hot Tap Water is, according to current scientific knowledge and practical experience, not a reliable preventive measure to control *Legionella (pneumophila)* if the hot water temperature is below 60°C. This can even result in increased Legionella numbers after heat shock treatment due to growth of *Legionella (pneumophila)* on the dead biomass (released after heat shock treatment).

Based on the scientific insights, the advice is to modify the risk factors for collective mains networks of priority institutions in relation to domestic hot water. The risk factors should no longer be referred to as 'growth', 'neutral' and 'death' but as 'risk of the presence of cultivable Legionella' and 'no risk of the presence of cultivable *Legionella*'.
Installations with hot water temperatures lower than 60°C at the hot water system, return pipe of the hot water system, mixing unit and tap point are then given the qualification "risk of presence of culturable *Legionella*".
Systems with hot water temperatures higher than 60°C at the hot water installation, return pipe of the hot water installation, mixing unit and tap point or installations where the standards set in NEN 1006 are reached through reheating, are then given the qualification 'no risk of the presence of culturable *Legionella*'.

If the hot water temperature is lower than 60°C in the return pipe, at the mixing unit or at the tap point, it is also recommended that, if favorable growth conditions occur, the possibility of legionella control through the application of thermal disinfection by means of heat shocks should be abandoned. Under these conditions, the passages in the table in section 5.2 in appendix 2 of the Regulation for the prevention of Legionella in drinking water and domestic hot tap water concerning thermal disinfection at certain temperatures and times are also lapsed.

Based on the scientific insights concerning thermal disinfection by means of heat shocks, no unequivocal advice can be given about the use of thermal disinfection as a control measure for locations where favourable conditions for *Legionella* growth occur and where the hot water temperature is ≥ 60°C. Therefore, two different recommendations are proposed, one of which can be implemented:

A. Let the passages in Table 4 of NEN 1006 concerning thermal disinfection by means of heat shocks at certain temperatures and standing times also lapse for situations in which the hot water temperature ≥ 60°C. After implementation of this advice, monitor intensively what the influence is on the numbers of *Legionella* in the hot water system, mixing unit and/or outlet pipe. If the observed that legionella numbers increase due to the expiration of this measure, then it is recommended that the lapsed passages to be reinstated.

B. For the time being, maintain the passages in Table 4 of NEN 1006 concerning thermal disinfection by means of heat shocks at certain temperatures and standing times if the hot water temperature is ≥ 60°C. At the same time, investigate how successful this control measure is in priority buildings where the measure is applied. Based on the results of the study, it can then be decided whether the measure can be maintained, should be modified, or should be dropped.

C. An adjustment in line with the above will affect the construction and installation of collective piping networks, as well as their use. The building managers, installation engineers and NEN norms subcommittee NEN1006 are the most important stakeholders in this respect. In particular, the elimination of thermal disinfection by means of heat shocks at priority settings, where the hot water temperature is 60°C or higher at the hot water supply, hot water system, return pipe, mixing device and aerosol forming tap point, has major consequences for the possibilities of control measures to be applied, if favorable growth conditions for the propagation of *Legionella* occur. That aspect could be factored into the choice of implementing the above advice A or B.

CHAPTER 4

Cold water systems versus hot water systems

4.1 Current legislation

A collective piping network consists of a drinking water piping network (cold water), a hot water preparation, in which the cold water is heated, and a hot water piping network and the tap points connected to it. Legionella regulations do not make an explicit distinction between cold water and tap water.
and hot water systems, so that both systems in priority settings are subject to the quality requirement for the number of legionella bacteria per liter and both systems must be an integral part of the risk analysis and management plan.

4.2 Reason to include topic in evaluation

In France, legionella legislation focuses only on hot water systems and not on cold water systems. Because the regulations on legionella prevention in France were formulated later than in the Netherlands, it is possible that new scientific insights have led France to decide to focus the regulations only on the hot water system of tap water systems.

4.3 Brief overview of scientific insights for 2001

Several studies published before 2001 reported the presence of culturable *Legionella (pneumophila)* in tap water systems (e.g., Wadowsky *et al.* , 1982, Groothuis *et al.* , 1985, Meenhorst *et al.* , 1985, Stout *et al.* , 1987, Farrell *et al.* , 1990, Stout *et al.* , 1992, Lück *et al.* , 1993, Zacheus & Martikainen, 1994). Usually only the hot tap water was sampled in these studies, but in a few studies the cold tap water was also sampled. These samples were also sometimes found to be positive for culturable *Legionella (*Wadowsky *et al.* , 1982, Farrell *et al.* , 1990).

4.4 Overview of scientific insights since 2001

4.4.1 *Legionella* in cold tap water samples abroad

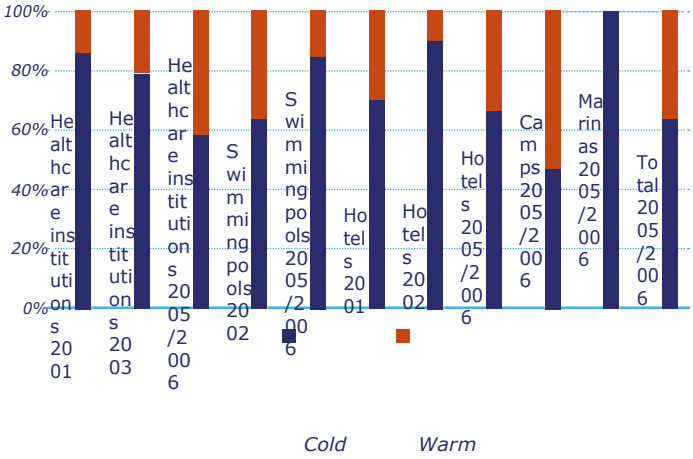
Since 2001, several additional studies have been published in which building hot and/or cold tap water
has been sampled for culturable *Legionella (pneumophila)* (Darelid *et al.* , 2002, Mouchtouri *et al.* , 2007, Veronesi *et al.* , 2007, Arvand *et al.* , 2011, Donohue *et al.* , 2014, Rodriguez-Martinez *et al.* , 2015). During a ten-year monitoring program in a Swedish hospital, no culturable *Legionella was* observed in the samples taken at cold water taps, but it was observed in samples taken at hot water taps (Darelid *et al.* , 2002). It was also observed that the water temperature of the cold tap water samples was always below 20°C. In countries with a relatively warm climate (Israel, Greece, Italy), culturable *L. pneumophila* were observed though
in cold tap water samples from hotels and/or hospitals

(Mouchtouri *et al.* , 2007, Veronesi *et al.* , 2007, Rodriguez-Martinez *et al.* , 2015). The Greek and Israeli study also determined the water temperature and it was above 22 to 23°C for most positive samples. However, it remains difficult to directly link legionella numbers to drinking water temperature, because propagation in a plant may occur on a hotspot that may be present in the installation (see Section 4.6), but the drinking water temperature was determined on a portion of the water that did not originate from this hotspot. Scientific studies focusing on hotspots and risk of legionella propagation were not found, by the way, and the risk of such hotspots therefore remains a knowledge gap in scientific knowledge.

A German study showed that culturable *Legionella* was present in cold water samples sampled at four different healthcare facilities in the town of Hesse (Arvand *et al.* , 2011). Thereby, in the distal part of the tap water system, more cold tap water samples were positive (40%) than hot tap water samples (23%). Also, the numbers of culturable *Legionella* were higher in the distal cold water samples than in the hot water samples. This German study shows that in a more temperate climate such as the Netherlands, cold water samples can therefore also be positive for culturable *Legionella* spp.

4.4.2 *Legionella* in cold tap water samples The Netherlands

In the Netherlands, it has also been investigated whether culturable *Legionella* in drinking water is present, but only in a few studies is also the distinction between cold and hot water made. In an extensive study of the presence of culturable *Legionella* in housing installations in the Netherlands, the cold and hot tap water were sampled separately at some positively tested installations and the results showed that in at least three of the sixteen homes culturable *Legionella* *were* found in the cold tap water (Oesterholt & Veenendaal, 2002). The RIVM analyzed Legionella data from priority institutions for the period 2001 through 2006 in 2007 (Versteegh *et al.* , 2007). These data were supplied by (at the time) the VROM Inspectorate and were obtained by the VROM Inspectorate from the building managers of priority institutions who had been asked during routine monitoring of *Legionella* in observed a norm violation (100 cfu/l culturable *Legionella* spp) in the tap water systems. The data show that 66 to 75% of the norm violations were observed in cold water pipes and 25 to 33% included cold tap water sampled at various taps of tap water systems from five sites (hospitals and hotels) (van Hoof *et al.* , 2014). Five of the ten cold tap water samples were positive for culturable *Legionella* and one of the ten hot tap water samples. The highest culturable legionella numbers were also found in the cold water samples. In addition, a study with a pilot tap water system that was fed with Dutch drinking water showed that L. anisa could multiply up to 1×105 cfu/l in the cold water part of the system that always had a drinking water temperature below 25°C (van der Lugt *et al.* , 2017, van der Lugt *et al.* , 2019). Finally, drinking water companies also routinely monitor *Legionella* spp in cold water sample taken from taps in buildings or fire hydrant after flow-through and these data are reported annually by the Environment and Transport Inspectorate. In 2019, an exceedance of the legionella standard (100 cfu/l) was observed in 5.1% of the samples (Anonymous, 2020), which is also shows that culturable *Legionella* is observed with some regularity in cold water samples.



in hot water pipes (Figure 4). In another study,

Figure 4. ***Legionella*** **norm violations (100 cfu/l) in cold and hot water pipes as found in the VROM-Inspectie surveys between 2001 and 2008. Source: Versteegh *et al.* , 2007.**

The RIVM has shared case histories and results of the *Legionella* Source Detection Unit (BEL) study showing that also in the Netherlands certain cases of *Legionella* illness were matched with the presence of the same strain in the cold water system of a tap water system. In addition, the results of the BEL study show that culturable *Legionella* is found with some regularity in the cold water system of a tap water system.

4.5 Conclusion scientific state of the art flushing tap water plant

Scientific studies on the presence of *Legionella* in tap water systems and case studies in the Netherlands show that culturable *Legionella* spp including *L. pneumophila* can be found in both the cold and hot tap water of a tap water system.

4.6 Knowledge and experiences from practice

There is a consensus among all respondents on this topic: the focus on both domestic hot water and domestic hot water in the regulations must be maintained. If only because in a sanitary installation hot and cold water always come together in a mixer and contamination from the cold water side can cause problems in the mixer itself and in the downstream part of the installation. Experience in caring for the disabled has shown that the most stubborn infections are the ones that cause the most problems. exceedances occur in cold water pipes. In most cases, incidentally, this involves *L.* nonpneumophila. From the survey conducted by ISSO in 2017 among parties involved in legionella prevention in tap water systems also showed that most of the problems in practice are linked to legionella growth in cold water pipes. Think of hotspots in the cold water part of the system that are arise from hot rooms, hot shafts and interactions with underfloor heating, where the temperature can sometimes rise above 30 °C. Another factor is that in newly built houses and buildings with low temperature heating (floor and wall heating) the installation of a cold water system without hot spots is becoming increasingly difficult. This literally leads to many "detours" and longer pipe lengths.

4.7 Recommendation to adjust regulations based on scientific insights

The current regulations Legionella prevention in drinking water and hot tap water focus on the cold and hot tap water part of the tap water system, as well as parts of the tap water system where risk of propagation of culturable *Legionella* spp may occur. From the analysis of the scientific publications and from the interviews with people with practical experience, it follows that the current scientific understanding and practical experience correspond to the current regulations concerning this point.

The advice based on the scientific evidence is therefore not to amend the current legislation in this respect and thus to continue to focus the regulations on the cold water part and the hot water part of the tap water system, both for the quality requirement and for the obligation to carry out a risk analysis and to draw up a management plan.

CHAPTER 5

Influence of flushing tap water system on *Legionella* in buildings

5.1 Current legislation

Article 37, paragraph 1 of the Drinking Water Decree refers to the Legionella Prevention Regulations (regulations to be drawn up by ministerial regulation) for the requirements set for carrying out a risk analysis. Appendix 2 of the Regulation for the prevention of Legionella in drinking water and warm tap water contains a summary of the requirements to be used for the risk analysis.
risk factors and qualification of risks. One of the measures listed (5.1.5) is that *'cold and hot water pipes that are not used for more than one week are flushed weekly. When flushing, water is tapped until 10 seconds after a stable temperature has been reached'.* This flushing measure is part of what the legislature considers thermal management.
Therefore, this flushing measure is in line with the risk mitigation factor as mentioned in 5.1.2. of Annex 2: *'The risk analysis shall take into account at least the following risk mitigation factors: (b) water temperatures between 20 and 25°C. Insofar as there is a maximum of one week of standstill and a good through flow'.*

5.2 Reason to include topic in evaluation

In the Netherlands, there is discussion among legionella experts, who are involved in daily practice with legionella control in tap water systems, about the effectiveness of flushing as a measure to reduce *Legionella* control. In certain publications, the effectiveness of flushing as a management measure has been challenged in relation to current scientific insights (van der Lugt et al, 2019) and practical experiences (Nuijten, 2019). Several members of the supervisory committee of the present project also indicated the effectiveness of flushing as a topic that should be looked at more closely. Based on the two aforementioned publications and response from several members of
the Guidance Committee, it is therefore unclear whether the Regulation on Legionella Prevention in Drinking Water and Hot Tap Water in terms of flushing as a control measure is still in line with current scientific insights.

5.3 Brief overview of scientific insights for 2001

Scientific studies published before 2001 have shown that water stagnation in parts of a tap water system can increase the growth of *Legionella (pneumophila)* compared to parts of the tap water system where there is no stagnation (e.g. Tobin *et al.* , 1981, Fisher- Hoch *et al.* , 1982, Ciesielski *et al.* , 1984). Based on those studies, flushing of tap water systems to prevent water stagnation is used as a management measure.

5.4 Overview of scientific insights since 2001

5.4.1 Drinking water with disinfection residue such as chlorine

More recent studies have confirmed that water stagnation in an existing tap water system can increase the numbers of *Legionella* in a tap water system and that by applying a flushing regime these numbers can be reduced again, provided a proper flushing regime is applied (Cristina *et al.* , 2014, Totaro *et al.* , 2018, Bédard *et al.* , 2019, Hayes-Phillips *et al.* , 2019, Nisar *et al.* , 2020). In addition, *Legionella* has been observed more frequently when buildings that were closed during the lockdown of the COVID-19 crisis were reopened and which is attributed to the stagnation of drinking water in the system during the lockdown (De Giglio *et al.* , 2020, Hozalski *et al.* , 2020).

Compared to flowing water, still water in a tap water system has lower concentration or no disinfection residue (Fisher-Hoch *et al.* , 1982, Wang *et al.* , 2012), lower water temperature (Patterson *et al.* , 1994), higher concentration of organic carbon (LeChevallier *et al.* , 1996, Wang *et al.* , 2012), lower dissolved oxygen concentration (Wang *et al.* , 2012), higher biomass concentration (Lauten- schlager *et al.* , 2010), different microbial community composition (Lautenschlager *et al.* , 2010, Dai *et al.* , 2018) and higher numbers of host protozoa (Wang *et al.* , 2015). These factors can all influence the growth of *Legionella (pneumophila)*. The different composition of
the microbial community - lower oxygen concentration and higher organic carbon concentration, biomass concentration and host protozoa - is likely caused by increased growth of microorganisms because growth is no longer inhibited by a disinfection residue or because
microorganisms are given longer time to absorb the more difficult
convert more degradable substances into drinking water. Additional scientific research in countries where drinking water with a disinfectant residue is distributed has shown
that the main mechanism of action of increased legionella infestation during downtime of drinking water in a tap water system is caused by the lower concentration or absence of a disinfectant residue (Hozalski *et al.* , 2020, Huang *et al.* , 2020, Martin *et al.* , 2020, Nisar
et al. , 2020). These studies were conducted in existing tap water systems or in a pilot tap water system where the experiments were conducted with piping material where biofilms had six years to develop before the experiments were conducted.

As for thermal management, flushing can potentially be applied as a preventive measure or as a curative measure. The scientific studies mentioned in the previous paragraph applied flushing regimes as a preventive measure, or an association was found between indirect flushing (e.g., frequent showering and infrequent showering) and Legionella. Studies in which flushing was used as a curative measure were not found. Based on the scientific literature that exists on *Legionella* in relation to stagnation and the effect of flushing to prevent stagnation, a committee of *Legionella experts* concluded that flushing can help control *Legionella* in building drinking water systems, but that no consensus can be obtained from the scientific studies as to what the best flushing strategy is (e.g., duration and frequency of flushing actions) and that it may vary from facility to facility (National Academies of Sciences, 2019).

5.4.2 Drinking water without disinfection residue

In the Netherlands, drinking water is distributed without a disinfection residue and with very low concentrations of degradable substances (van der Kooij & van der Wielen, 2014), which
the mechanism of action related to the disappearance of a disinfection residue during standstill does not apply to the Dutch situation. Therefore it is not possible to give results about the influence of standstill and flushing on *Legionella* in drinking water systems fed with chlorinated drinking water.
to translate for the Dutch situation. Only studies conducted with drinking water without a disinfection residue during distribution can be used to determine the extent to which flushing is a successful control measure for *Legionella* in Dutch drinking water systems. A recent review from December 2020 of scientific studies that
have been published on the influence of water stagnation and flushing
on growth of *Legionella* in tap water systems, has allowed

see that no studies have been published examining the impact of flushing or stagnation of drinking water on *Legionella* in tap water systems supplied with drinking water without disinfection residue (in other words non-chlorinated drinking water)(Nisar *et al.* , 2020).

However, in a U.S. study, the influence of stagnation, turbulent flow and laminar flow on the growth of *L. pneumophila* in a pilot tap water system, where 95% of the drinking water was recirculated (Liu *et al.* , 2006). Although the concentration of free chlorine was not determined, it is likely that under these conditions the disinfection residue of the drinking water was largely reacted away, causing the system was probably operating with drinking water with no or a very low concentration of a disinfectant residue. The results showed that the lowest numbers of *L. pneumophila* were found when the water was stagnant, while the highest numbers were found under turbulent flow. Therefore, the conclusion from this study was that stagnation is not a risk factor for growth of *L. pneumophila*.

However, several studies have been published that have shown that overnight stagnation of drinking water in existing tap water systems, which are fed with drinking water without a disinfection residue, can result in increased bacterial counts or active biomass in the drinking water (Lautenschlager *et al.* , 2010, Brewer *et al.* , 2018, Proctor *et al.* , 2018). The study by Brouwer *et al.* (2018) was conducted with citizen scientists on drinking water sampled at the kitchen faucet of houses in Amsterdam and showed that in some houses the bacteria numbers and active biomass in the drinking water from the plant were lower overnight after shutdown, while in other houses higher bacteria numbers and biomass concentrations were found. This shows that tap water plant factors play a role on the influence of standstill on microbiological water quality, but what factors these are has not been further investigated.

However, stagnation of drinking water in the tap water system can cause the temperature of the hot water to drop and that of cold water to increase during stagnation, which can put the water temperature in the range where *L. pneumophila* is able to reproduce (Rhoads *et al.* , 2016, Zlatanovic *et al.* , 2017, Jacobs *et al.* , 2018).

5.4.3 Dutch situation

Studies in which the effect of flushing on *Legionella* was investigated in Dutch tap water systems were not found. However, it was investigated to what extent culturable *Legionella* spp was more often present in showers and taps of people who contracted Legionnaires' disease after going on vacation than of people who contracted Legionnaires' disease but did not go on vacation (Verhoef *et al.* , 2004). The results showed that culturable *Legionella* spp was observed slightly more often in the tap water system of patients who had been on vacation (25.7%) than in tap water system of patients who had not been on vacation (15.7%). In addition, it was seen that showers of patients who had been on vacation also contained culturable *Legionella* spp more often (15.8%) compared to showers of patients who did not go on vacation (7.3%). However, in both cases the results were not statistically significant, probably due to the small sample size. In addition, information on how long the water stood still in the tap water system is missing, and the samples from the tap water system were taken after an infection with *Legionella* was identified. During the time from infection to identification of infection and sampling in the home, the people who went on vacation actively used their tap water system.

5.5 Conclusion scientific state of the art flushing tap water plant

In summary it can be concluded that most studies performed with drinking water with a disinfection residue show that water stagnation leads to increased Legionella numbers and that an effective flushing regime reduces Legionella numbers. Because the increase of *Legionella* and the effect of flushing in these studies is mainly caused by the presence or absence of a disinfection residue, these results cannot be translated to the Dutch situation where drinking water without a disinfection residue is distributed.
Based on the current scientific literature, no statement can be made about the extent to which weekly flushing of unused taps is a successful strategy to control culturable *Legionella* in drinking water from Dutch tap water system.

Knowledge and experiences from practice

One of the respondents indicated that relying on the effectiveness of weekly flushing is an uncanny starting point, possibly leading to a sense of false security, after all it is only effective if the pipe sections in question are more or less clean. Furthermore, we know that due to the use of certain plastic pipe materials, legionella contamination can be very persistent. Another respondent indicates that the requirement for weekly flushing (up to 10 seconds after reaching a stable temperature) in the Legionella Prevention Regulations makes the existing requirement in NEN 1006 more stringent. The question is whether this still adds anything and whether the requirement in NEN 1006 is not sufficient, because now it leads to confusion in practice.

It was also indicated that weekly flushing has a generic effect, but that it sometimes backfires. If the temperature does not fall sufficiently below 25°C in the outlet pipes, it is actually impossible to flush. Moreover, flushing is often a human activity and difficult to maintain. Automation can offer a solution here. Weekly flushing of little-used hot and cold water outlets is done in our healthcare facilities because it is a legal requirement. These actions produce few results and this causes frustration among the care personnel. It is flushing for the sake of flushing. Flushing with a thermometer does lead to a little more awareness among staff.

In new construction of homes, the specifications often require that the water system - once filled
– is regularly flushed and monitored for culturable Legionella. This seems to work fine in practice because upon completion, the best practice requirement of less than 100 cfu/l *Legionella* is almost always met.

5.7 Recommendation to adjust regulations based on scientific insights

Dutch legislation lists weekly flushing of drinking water systems as a measure against
organoleptic problems. In addition, flushing is also indirectly mentioned in the Regulation on Legionella prevention in drinking water and hot tap water in section 5.1.5 of Annex 2 (see 5.1). Based on the current scientific literature no statement can be made about the extent to which weekly rinsing of unused taps is a successful strategy to control culturable *Legionella* when the drinking water does not contain any disinfection residue, as is the case in the Netherlands. Flushing is used as a preventive control measure at several locations in the Netherlands and a number of practitioners indicated during the interviews that it is
is not always effective, is difficult to carry out, gives rise to frustration among personnel and can provide false safety. As an exception, preventive flushing of newly constructed tap water systems before delivery (in accordance with specifications) does seem effective.

The advice based on the scientific insights is to further investigate the possibility of no longer including flushing as a control measure against culturable *Legionella*, because from the scientific literature no evidence has been found that this measure is effective for Dutch drinking water without disinfection residue and because in practice the experiences vary, with adverse effects also being observed.
However, the advice remains to maintain flushing in the legislation (via NEN 1006) as a measure against organoleptic problems.

CHAPTER 6

Influence of material use tap water installation

6.1 Current legislation

In buildings in the sense of article 1, paragraph 1, of the Housing Act and in drinking water companies, collective water facilities and collective piping networks (insofar as they are not part of buildings), the Regulation on materials and chemicals for drinking and hot tap water facilities applies. These regulations are based on the Building Act 2012 for buildings and on the Drinking Water Decree in other situations.

Section 8(1) of the Regulations on Materials and Chemicals for Drinking and Hot Water Supply states that: *'All materials may be subject to laboratory examination, carried out in accordance with Appendix C to this regulation, with the aim of verifying compliance with the requirements of this regulation'*. In addition, Annex A of these regulations includes the following under section 2.2.7 'Investigation and assessment':

'To carry out the admission examination of plastics and rubber products, in accordance with Chapter 3 of the Regulations and Annex C, the following examinations should generally be carried out:

- Assessment of the recipe, testing against the positive lists of Annex B, determination of maximum allowable concentration (MTC)'s. For PVC and PE pipes a specification level of 0.1% (w/w) applies to the recipe, for rubber rings this level is set at 0.5% (w/w).
- A migration test.
- Review of organoleptic aspects.
- Establishing aftergrowth.

For products with a relatively small contact area for which, in accordance with part A, section 5 of the common approach for organic materials, a conversion factor < 0.01 d/dm can be established, a limited set of laboratory tests will generally suffice. The authorization tests required for these products are listed under the relevant product descriptions. If a product is not mentioned, then - at the discretion of the committee - the following aspects may apply:

- Assessment of the recipe, review against the positive list of Annex B, determination of MTCs.
- Calculate the expected concentration in drinking or hot tap water of substances subject to an MTC in accordance with Chapter 3 and/or 4 of Appendix C.
- Organoleptic aspects, if the product cannot be adequately removed (such as an adhesive).
- Aftergrowth aspects.'

Also included in Appendix A is section 2.3.2 of the 'Regulation on materials and chemicals for drinking and hot tap water supply':

'To carry out the admission examination of plastic films, in accordance with Chapter 3 of the Regulations and Annex C, the following examinations should generally be carried out:

- Assessment of recipe, review against Annex B positive lists, determination of MTCs.
- A migration test.
- Review of organoleptic aspects.
- Establishing after-growth aspects.

The testing of organoleptic aspects and the determination of after-growth aspects are not applicable to geomembranes.'

In Appendix C referred to in Appendix A, concerning growth potential of materials, the following is included about the methods and criteria that can be applied to determine aftergrowth by materials:

'For the determination of aftergrowth the standard NEN-EN 16421:2014 is applicable. NEN-EN 16421:2014 describes the test methods Biomass Production Potential (BPP), Biofilm Volume (VM) and Mean Dissolved Oxygen Depletion (MDOD).

For BPP, the assessment criterion is 1,000 pg ATP/ cm2.

If the evaluation criteria used in the VM and MDOD test methods provide an equivalent level of protection to the BPP evaluation criterion, then the test results obtained using the VM or MDOD method may be used. For VM, this is the assessment criterion of 0.05 ± 0.02 ml mucus volume/800 cm2.

No BPP criterion has yet been established for elastomers used as sealing materials in contact with drinking water. For the time being, the following assessment criteria apply
VM of 0.12 ± 0.03 ml mucus volume/800 cm2 and 0.20 ± 0.03 ml mucus volume/800 cm2 for sealing materials with large and small contact area with drinking water, respectively.'

6.2 Reason to include topic in evaluation

Over the past few decades, KWR Water Research Institute has done a relatively large amount of research into the influence of pipe material on biofilm formation and growth of Legionella. From
Those studies have shown that the type of pipe material can influence Legionella growth potential. A recent publication therefore noted that although certain pipe materials can cause increased growth of *Legionella* in (parts of) tap water systems, pipe material is not included as a risk factor in the Legionella Prevention Regulations (Nuijten, 2019). Nuijten (2019) further notes that rubber (EPDM) and soft plastic components do not belong in tap water systems or frequent need to be replaced.

If their application is unavoidable, their contact area should be limited as much as possible.
Several members of the supervisory committee for the present project also indicated that
pipe material is a possible contributing factor to the legionella problem in tap water systems and therefore the topic should be evaluated in relation to current scientific knowledge.

6.3 Brief overview of scientific insights for 2001

Piping materials can also influence the growth or death of *Legionella* in drinking water systems. In Dutch tap water systems, the following types of piping materials are most common: copper, PE (in the form of
PE-Xa, PE-Xb or PE-Xc), PVC-P, stainless steel (SS) and rubber. The literature review therefore focuses particularly on scientific studies in which the influence of these
materials on growth of *Legionella* and biofilm has been investigated. Prior to 2001, some sporadic studies had already appeared showing that culturable Legionella numbers on copper in contact with drinking water were lower than on chlorinated
PVC (PVC-C or PVC-U) or polybutylene (Schoenen & Wehse, 1988, Rogers *et al.* , 1994). It had also been observed that soft PVC (PVC-P), polyethylene (PE) various rubber types (bromobutyl, chlorobutyl, butyl, silicone, EPDM) and silicones increased the growth of *L. pneumophila (*Niedeveld *et al.* , 1986, Schoenen & Wehse, 1988).

6.4 Overview of scientific insights since 2001

6.4.1 Plastic and rubber materials

The results of studies that investigated the influence of piping materials on propagation of *Legionella* showed that plastic and rubber piping materials, especially polyethylene (PE), polypropylene (PP), polybutylene (PB), soft PVC (PVC-P) and synthetic rubber (EPDM) led to increased numbers of *Legionella (pneumophila)* (van der Kooij *et al.* , 2002, van der Kooij *et al.* , 2005, van der Kooij & Veenendaal, 2007, Moritz *et al.* , 2010, Proctor *et al.* , 2017, Learbuch *et al.* , 2019). These studies also showed that the plastic materials PVC-C and PVC-U can be used to many
resulted in lower legionella numbers than the other plastic materials or EPDM rubber.

Some of these studies also included silicone materials, with one study finding that silicone rubber was barely growth-promoting for *L. pneumophila* (van der Kooij & Veenendaal, 2007), while another study found certain silicone types to be strongly were growth-promoting for *L. pneumophila (*van der Kooij *et al.* , 2002).

Growth-promoting substances leaking to the surface of the material or drinking water are a direct cause of the increased growth of *Legionella* or other microorganisms on the plastic materials, where it has been seen that the more growth-promoting substances are released by a piping material (PVC-P > EPDM > PE/PP/PB > PVC-C/PVC-U), the the higher the biofilm concentration and legionella numbers (van der Kooij *et al.* , 2002, van der Kooij & Veenendaal, 2007, Learbuch *et al.* , 2019). In addition, some plastic materials can react with a disinfection residue, which lowers the disinfection residue in the water, reducing the inhibition of bacterial growth. However, this reaction of chlorine with plastic materials is slow, so these effects are not significant (Cullom *et al.* , 2020).

6.4.2 Metal materials

In addition to plastic and rubber materials, a number of studies have also examined the influence of stainless steel and copper piping on the growth of Legionella. For stainless steel, it was generally observed that stainless steel showed low numbers of *Legionella* compared to PVC-P, EPDM and/or PE (van der Kooij *et al.* , 2002, van der Kooij & Veenendaal, 2007, Assaidi *et al.* , 2018, van der Kooij *et al.* , 2020). However, one study showed that the numbers of culturable *L. pneumophila* in a pilot tap water system with stainless steel pipes were similar to the legionella numbers observed in the installation with PE-X pipes (van der Kooij *et al.* , 2005). For copper, the results from the different scientific studies are inconsistent. A number of publications show that under controlled conditions in the laboratory copper has a protective effect against *Legionella* and that Legionella numbers in drinking water in contact with copper are lower than observed for other pipe materials (van der Kooij *et al.* , 2005, Proctor *et al.* , 2017, Assaidi *et al.* , 2018, Learbuch *et al.* , 2019).

On the other hand, a number of other studies have shown that under laboratory-controlled conditions copper results in similar or increased legionella numbers compared to stainless steel or PVC-C/PVC-U (van der Kooij *et al.* , 2005, Buse *et al.* , 2014, Giao *et al.* , 2015, van der Kooij *et al.* , 2020). Field studies, in which drinking water samples from tap water systems of homes and buildings were tested for *L. pneumophila* were investigated showed a similar picture. The results of a Danish study showed that lower numbers of *L. pneumophila* were found in tap water systems made of copper than of stainless steel (Pringler *et al.* , 2002), while a German study showed that copper tap water systems were more often positive for culturable *Legionella* than stainless steel or plastic materials (Mathys *et al.* , 2008).

Both stainless steel and copper do not secrete growth-promoting substances on which microorganisms can multiply (Cullom *et al.* , 2020), which explains why stainless steel generally does not lead to increases in Legionella. However, corroded iron or steel has been shown to lead to increased numbers of *Legionella* (van der Lugt *et al.* , 2017, Cullom *et al.* , 2020, van der Kooij *et al.* , 2020), probably because (i) *L. pneumophila* requires iron as a nutrient for growth and corroded iron results in release of iron ions and (ii) corrosion leads to a rougher surface and certain degradable substances can accumulate on the rougher surface. A rougher surface and accumulated degradables in a tap water system result in a higher biofilm concentration, which indirectly leads to higher legionella numbers. In addition, corrosion creates positively charged surface to which negatively charged biodegradable organic carbon bind, immobilizing these substances and giving microorganisms in the biofilm all the time they need to degrade these substances, which will also lead to higher biofilm concentrations. It is possible that the similar legionella numbers on stainless steel and PE-X materials in the study by Van der Kooij *et al.* (Van der Kooij *et al.* , 2005) was therefore caused by corrosion of stainless steel in the study, but iron corrosion was not quantified.

A plausible explanation why lower Legionella numbers are found with copper than with other pipe materials is that copper has an antibacterial effect on microorganisms and *Legionella (*Van der Kooij *et al.* , 2005, Cullom *et al.* , 2020). One explanation why other studies found no or opposite effect of copper on *Legionella* may be because release of the antibacterial copper ions to water decreases as the outer layer of copper has become oxidized (Van der Kooij *et al.* , 2005), reducing the concentration of free copper ions in copper pipe material. In addition, other water quality aspects (e.g. pH, concentration of other metal ions, concentration and composition of natural organic matter) also plays a role in neutralizing copper as an antibiotic (reviewed in Cullom *et al.* , 2020). When the concentration of free copper ions in the water becomes lower and the positively charged corrosion layer on the copper pipe binds additional organic material, the biofilm concentration (and related numbers of *Legionella*) can become higher, as has also been observed for corroded iron.

6.4.3 Dutch situation

A significant portion of the laboratory-controlled studies and field studies described above were conducted with drinking water without a disinfectant residue (Niedeveld *et al.* , 1986, Pringler *et al.* , 2002, Van der Kooij *et al.* , 2002, Van der Kooij *et al.* , 2005, Van der Kooij & Veenendaal, 2007, Mathys *et al.* , 2008, Moritz *et al.* , 2010, Learbuch *et al.* , 2019, Van der Kooij *et al.* , 2020), as is also distributed in the Netherlands.

The studies conducted in the laboratory with Dutch drinking water show that PE, PP, PB, PVC-P and EPDM rubber, unlike PVC-C, high quality stainless steel, copper and silicone rubber, promotes the growth of *L. pneumophila (*Van der Kooij *et al.* , 2002, Van der Kooij *et al.* , 2005, Learbuch *et al.* , 2019). In this regard, the maximum legionella numbers in the biofilm are strongly related to the biomass production potential of the material. Practical research on copper mixers in the Netherlands showed that the highest numbers of *Legionella* spp were found on the rubber parts of the faucet from tap water systems that were regularly positive for culturable *Legionella*. In this process, Legionella numbers on rubber were ten times higher than on the copper parts, while the swabbed area of rubber was smaller than of copper (van Hoof *et al.* , 2014). A recent study showed that copper also showed increased growth of *L. pneumophila* in a biofilm monitor that was flushed three times per hour compared to PVC-C, high quality stainless steel and the negative control glass (Van der Kooij *et al.* , 2020).

It was notable that increased biofilm formation and legionella growth on copper was observed from the beginning, whereas this was not observed in previous studies under semi-stagnant conditions or in a pilot plant (Van der Kooij *et al.* , 2002, Van der Kooij *et al.* , 2005, Learbuch *et al.* , 2019).

The authors suggest that this difference is because the concentration of copper ions, which act bacteriostatically or bactericidally against *Legionella,* remain low in the drinking water from the biofilm monitor, because the drinking water in the biofilm monitor is changed three times per hour (Van der Kooij *et al.* , 2020). If this hypothesis is correct, regular flushing of drinking water in copper pipes could possibly result in *Legionella* being able to grow to higher numbers in the biofilm. Incidentally, the applied flushing regime of three times per hour in the biofilm monitor is not realistic for drinking water practice and the results were obtained with a pilot plant, in which downtime occurs for eight hours per day (Van der Kooij *et al.* , 2005) and from cranes from full-scale plants (Van Hoof *et al.* , 2014) are more representative of real-world conditions.

The scientific studies show that with the set assessment criteria for the growth potential of materials in the Regulation on Materials and Chemicals for Drinking Water and Hot Water Supply, materials may be used in tap water systems that can lead to increased growth of *L. pneumophila* in the tap water system. The experiences in practice also show that legionella problems seem to be more common in tap water systems made of plastic materials.

The Biofilm Volume (VM) and Mean Dissolved Oxygen Depletion (MDOD) method are too insensitive to distinguish materials that may strongly promote growth of *L. pneumophila* (e.g., PE) from materials that do not or barely promote growth of *Legionella* (e.g. PVC-C, copper, stainless steel)(Van der Kooij & Veenendaal, 2007). This results, for example, in all PE materials offered for the VM and/or MDOD method meeting the assessment criterion for the VM and MDOD method (personal communication Dr. Christiane Schell). The assessment criterion for the Biomass Production Potential (BPP) method (1,000 pg ATP/cm2) is derived from the assessment criterion for the VM method (van der Wielen, 2011).

However, the relationship between biofilm concentration and the legionella growth potential of materials in contact with Dutch drinking water has shown that at a biofilm concentration of 1,000 pg ATP/cm2 (the current standard for growth potential of materials determined with the BPP-test) relatively high numbers of *L. pneumophila* can be found (~ ^{104} cfu/cm2; Figure 5)(Van der Kooij & Veenendaal, 2011, Van der Kooij *et al.* , 2017, Learbuch *et al.* , 2019). The ratio of *L. pneumophila* in the biofilm (in cfu/cm2) to *L. pneumophila* in the water (in cfu/l) under semistagnant conditions (weekly refreshment) later showed that ^{104} cfu/cm2 *L. pneumophila* in the biofilm corresponds with ^{105} cfu/l in drinking water (Van der Kooij & Veenendaal, 2007). In order to find *L. pneumophila* in drinking water below ^{104} cfu/l to be maintained under these semistagnant conditions, the numbers of *L. pneumophila* in the biofilm should therefore be below ^{103} cfu/cm2.

According to the equation of Figure 5, it means that the growth potential of the material should be below 400 pg ATP/cm2.

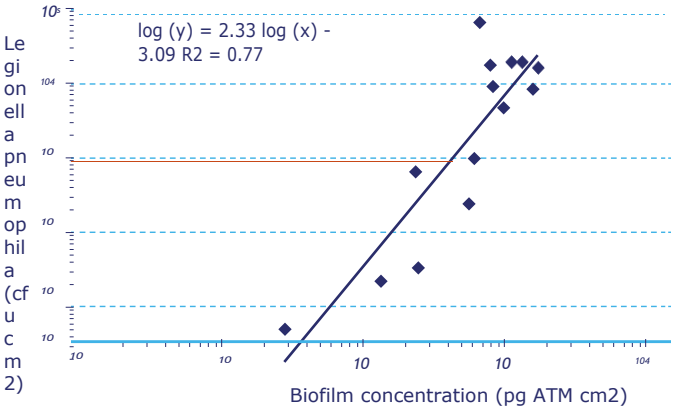


Figure 5. **The relationship between biofilm concentration in pg ATM per cm2 and the number of colony forming units (cfu) of *L. pneumophila* per cm2. The comparison of the correlation and the strength (R2) of the correlation is shown in the figure. The red line indicates at which biofilm concentration the culturable legionella numbers remain below 1,000 cfu/cm2. Source: Van der Kooij & Veenendaal, 2011, Van der Kooij *et al.* , 2017, Learbuch *et al.* , 2019.**

Table 3. **The minimum, maximum and average BPP values (in pg ATP/cm2) of various plastic and rubber materials. Data are from Van der Kooij (2002); Van der Kooij *et al.* (2003); Van der Kooij *et al.* (2006); Van der Kooij & Veenendaal (2007); Bereschenko (2013); Van der Wielen & Bereschenko (2016); Learbuch (2018).**

Material	Number tested	Minimum BPP	Maximum BPP	Average BPP
PVCU/C	34	5	619	149
PE	81	163	7352	1395
PB	2	364	836	
PP	5	336	732	537.4
Teflon	6	64	271	152
Silicone	12	325	32884	5867
Rubber nature	2	12735	13872	
EPDM	5	2475	45887	16921
PVCP	13	13395	48061	30287

Since the 1990s until now, the BPP values of various plastic and rubber materials have been determined within various public projects (Table 3). From the data of this table, it follows that PVC-U/C and Teflon mostly have BPP values lower than 400 pg ATP/cm2, that the BPP values of PE, PB, PP and silicones are both lower and higher can be greater than 400 pg ATP/cm2 and that rubber and PVC-P have BPP values that are always greater than 400 pg ATP/cm2.

In addition, despite the Regulations on Materials and Chemicals for Drinking Water and Hot Water Supply, materials which do not meet the above criteria for regrowth (e.g. shower pipes made of PVC-P) are regularly used in collective tap water installations. For example, various stores offer materials for tap water installations which do not contain a recognized quality declaration in accordance with the Regulations on Materials and Chemicals for the Supply of Drinking Water and Hot Water. A shower pipe is incidentally the last piece of a tap water installation, which is usually not supplied with hot water above 55°C, but with water around 37°C, the ideal growth temperature for *L. pneumophila* (National Academies of Sciences, 2020). In addition, the water from the shower hose is sprayed directly to the user. The use of piping material for the shower hose with strong growth-promoting properties for *Legionella (pneumophila)* may therefore pose an increased risk of propagation and spread of *Legionella (pneumophila)*. The source and contact investigation of *Legionella* has also shown that patient strains of *L. pneumophila* were related to the presence of the same strain in the biofilm on the shower hose.

6.5 Conclusion scientific state of the art material use tap water plant

Thus, the current scientific state of the art on the influence of piping materials on the growth of *L. pneumophila* in drinking water systems shows that piping materials can have an important influence. Application of
particularly PVC-P, EPDM rubber, some silicone types and PE materials in a tap water system can promote the growth of *Legionella* and lead to increased numbers of culturable *Legionella* in the tap water system. Also, it appears that copper over time can also result in increased numbers of *Legionella* in a tap water system
with copper pipe relative to stainless steel, certain silicone rubber type or PVC-C/PVC-U, but these numbers do remain generally lower than those observed with the other plastic pipe materials.

6.6 Knowledge and experiences from practice

From the interviews with people who have knowledge and experience of practical situations, a number of points were made about risk of material use. For example, it was indicated that with good thermal management, pipe materials play a minor role. However, materials do play an important role with mixing valves and downstream facilities because that is where the temperature is in the growth range for *Legionella*. This point is also recognized by the respondents from the disability care where many Legionella problems occur mainly in cold water pipes. In many collective tap water systems, the legionella problems are directly linked to the use of plastic piping materials.

Virtually all certified Legionella consultants will indicate that piping materials play an important role in the growth of biofilm and *Legionella*. Copper seems to perform better than some plastics in this respect. Incidentally, 'bad' materials are still used in new construction situations. There are many systems that do not have a recognized quality declaration according to the Regulations on Materials and Chemicals and yet are still being used.
applied. The influence of material choice on legionella growth should receive more attention. For example, it is not yet part of the risk qualification. In the existing ISSO 55.1, materials (indeed) do not come back as distinguishing in the risk analysis.

ISSO advocates the use of products with a recognized quality declaration, to the extent that such a declaration is available for a product, as this is not always the case. Another point of attention in the selection of pipe materials is the effect of ageing of these materials on the
biofilm formation and propagation of *Legionella*. The question is whether we have enough knowledge in that regard.

The position should be that products for which a recognized quality declaration is available should also be used in practice. Other products without such a declaration should then be banned and this should be enforced. For many composite products, however, there is still no recognized quality declaration5. This is true of most pipe materials, but the problem lies mainly with the range of fittings used in pipe systems. This applies, for example, to heat pumps and shower heat recovery units. These are new systems that do not yet have a recognized quality declaration, so the installer has no idea what materials have been used and what the effects of these are. On the other hand, there is pressure on installers to apply these systems because a certain energy performance coefficient (EPC) value must be met. It is also not always clear to installers exactly what a certification of a product refers to.

One of the respondents opted to differentiate in terms of material use within priority institutions. For example, for high-priority (care) institutions, there should be a ban on the use of materials with a
high biofilm formation potential. In such institutions, it should also be mandatory to replace shower hoses and shower heads every three years. Here it does appear to be possible to distinguish between good and bad shower hoses. At such a point, the industry also needs coercion from legislation to design and market safer products.

5BRL-K610/04 is an assessment guideline linked to the product certificate for thermostatic mixing valves such as sanitary thermostatic mixing valves for domestic use and safety valves in (health) care institutions.

Based in part on climate change and higher ambient temperatures, two respondents called for more attention to structural details of pipes, fittings and connections (tighter design of installations) and
a (voluntary) 'clean design label'. This involves the type of materials, but also the finish of materials (roughness) and the constructive details of fittings and connections (seams, corners, holes). Finally, it also appears
During construction, gains can be made by paying more attention to hygienic aspects related to the handling of pipe sections. Pipe sections are often left uncovered at the construction site for long periods of time so that dirt can accumulate in
the pipe. Incidentally, this is already a requirement stated in NEN 1006 (regulations 3.1.10 and 3.1.11) and these regulations are further elaborated in Water Worksheet 1.4 I (Hygienic working).

6.7 Recommendation to adjust regulations based on scientific insights

The current regulations, in which materials may be used that comply with the Regulation on Materials and Chemicals for Drinking Water and Hot Water Supply, are not in line with the current state of scientific knowledge regarding the influence of piping materials on the growth of *Legionella*. In addition, the interviews revealed that it is also the experience of practitioners that culturable *Legionella* is more often found in installation with plastic pipes or components made of PE, PVC-P or EPDM rubber.

The advice based on the scientific evidence is to include in the legislation that the biomass production potential (BPP) - determined using the BPP method described in NEN-EN 16421:2014 - of the piping materials to be used in new construction and/or renovation of tap water systems of priority buildings shall not exceed 400 pg ATP/cm2.

An adjustment in line with the above will affect system designers, builders and installers.

CHAPTER 7

Regulatory focus on culturable *Legionella* spp or *L. pneumophila*

7.1 Current legislation

These rules apply only to priority institutions.

Article 36, paragraph 1 of the Drinking Water Decree states that *'drinking water and hot tap water contain less than 100 colony-forming units of legionella bacteria per liter of the species of legionella bacteria to be determined by ministerial regulation. The regulation referred to in the previous sentence may include an equivalent of the permissible number of legionella bacteria per liter included in the previous sentence'. Article 41, first paragraph, of the Drinking Water Decree states that 'If the drinking water, as referred to in article 36, first paragraph, contains more than 1000 colony-forming units of legionella bacteria per liter, the owner of the collective water supply or the collective piping network concerned shall immediately and fully inform the inspector. The inspector may determine that the owner shall immediately and fully informs and advises them on the measures to be taken by them to protect their health'.*

In article 4, paragraph 1, of the Regulation Legionella prevention in drinking water and warm tap water, the following
legionella species to which the quality requirement referred to in Article 36 of the Drinking Water Decree applies.
'L. anisa, L. birminghamensis, L. bozemanii, L. cincinnatiensis, L. dumoffii, L. erythra, L. feeleii, L. gormanii, L. hackeliae, L. jordanis, L. lansingensis, L. longbeachae, L. maceachernii, L. micdadei, L. oakridgensis, L. parisiensis, L. pneumophila, L. sainthelensi, L. tusconensis, L. wadsworthii and L. waltersii.'

This therefore involves, in addition to *Legionella* pneumophila, other so-called *Legionella* non-pneumophila species that are also associated with disease in humans.

The following is added in paragraph 2 and paragraph 3:

2. If, when the method referred to in Article 7 is applied, it is found that the water contains less than 100 colony-forming units of legionella bacteria per liter, then assumed that the legionella species listed in the first paragraph are present in the water in numbers less than 100 colony-forming units per liter'.

3. If, when the method referred to in Article 7 is applied, it is found that the water contains 100 or more colony-forming units of legionella bacteria per liter, it shall be assumed that the species of legionella bacteria mentioned in paragraph 1 are present in the water in numbers greater than or equal to to 100 colony-forming units per liter, unless evidence to the contrary is provided.

These additions are necessary because the analysis method mentioned in article 7 can also be used to detect cultivable legionella species which are not mentioned in article 4, paragraph 1. Incidentally, in article 7 the culture method according to NEN-EN_ISO 11731 or an equivalent method is included as a requirement.

7.2 Reason to include topic in evaluation

The discussion of whether *Legionella* regulations should focus on culturable *L. pneumophila* or all cultivable legionella species has been playing out in the Netherlands for some time. Ten to fifteen years ago, the drinking water industry called on the Ministry of Housing, Spatial Planning and the Environment to modify the regulations to focus exclusively on *L. pneumophila*. The ensuing discussion revealed a difference of opinion at the time among legionella researchers as to what extent that position is supported by science. In other Western European countries, some countries' legionella regulations also focus on culturable *Legionella* spp (e.g., Great Britain, Germany), but in some other countries, legionella regulations focus only on culturable *L. pneumophila* (e.g., the Flemish part of Belgium and France). It is therefore useful to examine whether the focus on culturable *Legionella* in the Dutch regulations Legionella prevention is still in line with current scientific insights. A number of members of the guidance committee also indicated that this issue should be a priority for an evaluation.

7.3 Brief overview of scientific insights for 2001/2011

Scientific publications concerning the public health risk of *L.* nonpneumophila species, whether the presence of *L. pneumophila* is masked by growth of *L.* nonpneumophila species on the selective culture medium and whether *L.* nonpneumophila is a good indicator organism for *L. pneumophila* are only very sporadically present from before 2001. Thus, the scientific insights described in this chapter are primarily based on publications after 2001, but the following section also includes pre-2001 literature in some locations, to provide the most complete overview possible give. In 2011 the Regulation on Legionella Prevention was expanded to include a specification of species and this expansion was based on a report by Brandsema & Schalk, 2010. With respect to this expansion, 2011 was taken as the benchmark.

7.4 Overview of scientific insights since 2001/2011

7.4.1 Introduction

The discussion whether regulations should focus on culturable *Legionella* spp or only on culturable *L. pneumophila* has been going on in the Netherlands for several years among the Legionella experts and has also produced some papers. The discussion focuses on the following research questions:

1. Is the health risk from pathogenic legionella species other than *L. pneumophila* so great that it is necessary to take management measures against all pathogenic legionella species?
2. To what extent does the presence of *L.* nonpneumophila species on agar culture medium according to ISO 11731 the presence of *L. pneumophila* and therefore risky situations with *L. pneumophila* are missed?
3. To what extent is *L.* nonpneumophila an indicator organism for the presence of *L. pneumophila* in tap water systems?

As described earlier, the Regulations were amended in 2011 to explicitly mention all pathogenic legionella species in the legislation. The reason was that the legislative body anticipated a new method that would appear on the Dutch market (the so-called Legionella chip), which could measure all Legionella species in the water at once. This Legionella chip was marketed for a short time, but has been taken off the market again since a number of years and is no longer available. The remainder of this paragraph examines the current scientific knowledge concerning the three research questions posed above.

Within Section 7.4, we examine, among other things, what the current scientific knowledge is regarding the number of disease cases caused by *L.* nonpneumophila, and how that compares to other pathogens that may also occur in drinking water, but also explicitly include whether monitoring of *L.* nonpneumophila is an indication of *L. pneumophila* and/or that the management of the facility is inadequate against *L. pneumophila* and to what extent the presence of *L.* nonpneumophila can mask the detection of *L. pneumophila*. Thus, the final conclusion and opinion regarding this section is based on and substantiated by the scientific knowledge regarding all three of these issues.

7.4.2 Infections by different legionella species in the Netherlands and abroad

Legionella pneumonia (Legionnaires' disease) is a notifiable disease in the Netherlands. The number of reported cases of Legionella pneumonia from 2012 to 2019 is shown in Figure 6. It follows from this figure that during the period 2012 to 2017/2018, an increase in the number of reported cases of Legionella pneumonia in the Netherlands was observed, to almost 600 cases in 2018, of which more than 400 patients contracted the disease in the Netherlands. The diagnosis of *Legionella* in patients with pneumonia is in most countries mainly performed with the relatively simple and rapid urine antigen test (National Academies of Sciences, 2019). A disadvantage of this urine antigen test is that it primarily detects infections with *L. pneumophila* serogroup 1 reliably detects. Some urine antigen tests also detect other serogroups of *L. pneumophila*, although with lower sensitivity. The detection of disease by other variants requires clinical material from the patient's deep lungs (such as sputum or bronchoalveolar lavage). Since many patients with legionella pneumonia do not give up sputum, diagnostic possibilities are limited and many diagnoses (especially *L. pneumophila* nonserogroup1) will be missed. As a result, it is generally assumed that the number of cases of legionella pneumonia diagnosed is an underreporting of the actual number of cases (National Academies of Sciences, 2019), because other serogroups of *L. pneumophila* (serogroups 2 through 14) and other legionella species can also cause legionella pneumonia (Brandsema & Schalk, 2010).

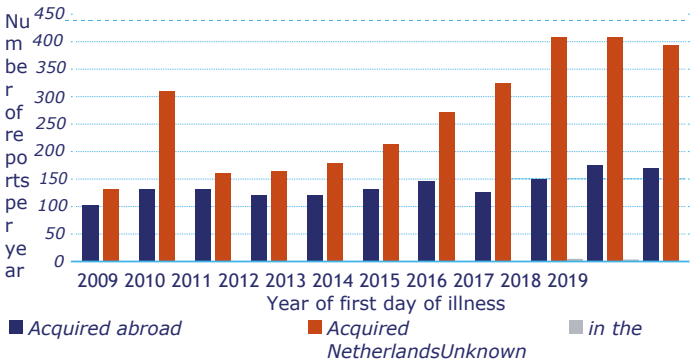


Figure 6. **The number of reports of pneumonia from *Legionella* contracted in the Netherlands (red) or abroad (blue) per year in the period 2009-2019.** The patients represented by the blue bar were in the period of 2-10 days before first day of illness abroad and most likely contracted the infection abroad. However, some of these patients may also have become infected in the Netherlands. Source: RIVM, Osiris.

Prior to 2011, the genus *Legionella* contained at least fifty different described species, of which 21 species have been described in relation to disease cases (Brandsema & Schalk, 2010). Since 2011, more Legionella species have been identified, so that today there are over sixty described Legionella species, of which 28 are associated with disease (Reukers *et al.* , 2020). In Europe, usually 90 to 98% of reported cases of legionellapneumonia caused by *L. pneumophila* and 2 to 10% of cases by legionella species other than *L. pneumophila* (Ricketts & Joseph, 2007, von Baum *et al.* , 2008, von Baum & Lück, 2011, Beauté & Network, 2017). For the period 2011 to 2015, we also described which legionella species were present in Europe were found when legionella infection was confirmed with culture (Beauté & Network, 2017). *L. pneumophila* serogroup 1 was found in 3020 of the 3645 (82.9%) positive culture tests, while 491 (13.5%) of the cultured legionella strains belonged to *L. pneumophila* serogroups 2 through 14 or an unknown serogroup of *L. pneumophila*. In addition to *L. pneumophila*, *L. longbeachae* (35 cases, 1.0%), *L. bozemanii* (15 cases, 0.4%), *L. micdadei* (12 cases, 0.3%), *L. anisa* (2 cases, 0.1%), *L. dumoffi* (2 cases, 0.1%), *L. cincinnatiensis* (1 case, < 0.1%), *L. macaechernii* (1 case, <0.1%) and *L. sainthelenis* (1 case, <0.1%) found very sporadically with culture. The remaining 65 positive culture results were not identified (38 cases, 1.0%) or belonged to other legionella species (27 cases, 0.7%) that were not specified.

These culture results show that by relying solely on the urine antigen test, cases of disease caused by *L. pneumophila* serogroup 2 through 14 are missed. *L.* nonpneumophila species are also sporadically detected in patients, but this incidence is also very low based on culture results. It is important, however, to provide some nuance. First, clinical material is not obtained in many patients, which means that the deployment of culture is often not possible. Also, certain *L.* nonpneumophila species *(L. birminghamensis, L. cherrii, L. cincinnatiensis, L. dumoffli, L. longbeachae, L. santicrucis, L. steigenvaltii)* to grow less well on the selective agar medium (Lee *et al.* , 1993). Furthermore, in countries where PCR testing is more frequently used for diagnosis, more cases of *L.* nonpneumophila are also observed (National Academy of Sciences, 2019).

It should also be noted with these numbers that this is the total number of legionella cases over the period 2011 to 2015 in Europe. The proportion of these legionella cases caused by drinking water is not known. In conclusion it is difficult to make comparisons between European countries from these data, because the diagnosis and attention to *Legionella* varies from country to country. Published data from a number of individual European countries, Japan, and the United States show similar observations (a review of scientific articles on this subject can be found in a recent publication by the National Academies of Sciences, Engineering and Medicine (National Academies of Sciences, 2019). In this regard, most *L.* nonpneumophila species are found mainly in patients with severely weakened immune systems (Muder & Victor, 2002, Cunha *et al.* , 2016).

In the Netherlands, *Legionella* case-finding is reported annually by the RIVM in the report 'Annual report surveillance of influenza and other respiratory infections in the Netherlands 20xx/20xx' available through the RIVM website. This section describes the results of the past five years (2015 through 2019) extracted from the last published RIVM report (Reukers *et al.* , 2020), supplemented with information obtained from RIVM regarding *L. anisa* over a longer time period and more information regarding the patients who contracted an infection with *L.* nonpneumophila. Over the past five years, a positive culture for *Legionella* was obtained from patient material in 479 cases of illness (19% of total cases of illness) and in 443 of culture positive results in the period 2015 through 2019, an isolate was also available for typing by species and serotype. 414 of these 443 isolates (93.5%) belonged to *L. pneumophila*, of which 379 (85.6%) belonged to serogroup 1 and the remaining 35 isolates (7.9%) belonged to serogroup 2-14 or an unknown serogroup. 26 of these 443 isolates (5.9%) belonged to *L.* nonpneumophila, of which 20 isolates (4.5%) belonged to *L. longbeachae*, 2 isolates (0.45%) to *L. bozemanii*, 2 isolates (0.45%) to *L. anisa*, 1 isolate (0.23%) to another legionella species and 1 isolate (0.23%) could probably not be confirmed. Thus, these culture results from the Netherlands also show that by relying solely on the urine antigen test, particularly *L. pneumophila* serogroup 2 through 14 are missed as well as *L. longbeachae*. Other *L.* nonpneumophila species are detected only very sporadically in the Netherlands and, based on culture results, this incidence is therefore also very low in the Netherlands.

Additional information from RIVM indicates that three patients with *L. anisa* were reported during the period 2008 through 2019. One of these three patients died. In two of these three patients, the infection was contracted in the hospital and these two patients also had an underlying condition. The third patient had a severely weakened immune system and it has remained unclear where this patient contracted the infection, although it could be concluded that this patient did not have it in the hospital has incurred. In the period 2017-2019, in addition to *L. anisa* another 22 patients reported with an infection with *L.* nonpneumophila, nineteen of which had *L. longbeachae*. Two of these nineteen patients died and ten of the nineteen patients had an underlying disease. Finally, three patients had become ill from another legionella species. One of these last three patients possibly involved a hospital infection, another patient probably contracted the infection abroad, and the third patient contracted the infection in the Netherlands but outside the hospital. Two of these three patients died and all three patients had an underlying disease.

In addition, it has been found in the Netherlands that in three-quarters of pneumonia cases in hospital the pathogen is not identified (Dijkstra *et al.* , 2008) and although a large number of microorganisms other than *Legionella* also have a can cause pneumonia, a proportion of these patients could also have been affected by undetected infection with a particular legionella strain. Based on data from pneumonia in 2015, it is estimated that 1,000-1,400 patients with legionellapneumonia are admitted to hospital (i.e., two to three times higher than currently diagnosed) and that, in addition, another 7,000-10,000 patients with legionellapneumonia consult the general practitioner (Vermeulen *et al.* , 2019). This could be interpreted that these undetected legionella pneumonia cases are caused by *L.* nonpneumophila, but this conclusion is not supported by the available data. First, it should be noted that the number of undetected cases of Legionella pneumonia is a rough and therefore somewhat unreliable estimate. In addition, patients who report to the general practitioner are not tested for *Legionella,* so from these patients it cannot be indicated which Legionella species is responsible for the Legionella pneumonia.

Hospital physicians apply the "NVALT-SWAB guideline Communty Acquired Pneumonia" for patients admitted to the hospital with pneumonia. This guideline states that only patients with a severe community acquired pneumonia are tested for *Legionella,* so it is possible that patients admitted to the hospital with Legionella pneumonia may not be tested for *Legionella*. As previously reported in a proportion of patients in whom the urine antigen test for *L. pneumophila* serogroup 1 is negative, *Legionella* was found via culture or PCR, with most of those cases of legionella pneumonia is caused by *L. pneumophila* serogroup 2-14. The same observations are also made in countries such as Denmark where diagnosis with culture and PCR is more intensive than in the Netherlands and where analysis of the culture/PCR data led to the conclusion that at least more than 90% of cases of legionellapneumonia were caused by *L. pneumophila (*Svarrer & Uldum, 2012). Therefore, there is a consensus within the scientific community that more than 90% of legionella pneumonia cases are caused by *L. pneumophila*.

Several studies in the Netherlands have shown that *L.* nonpneumophila is found much more frequently in Dutch drinking water than *L. pneumophila* (Van der Lugt *et al.* , 2019; Van der Kooij *et al.* , 2007). In these studies, 83 to 97% of *Legionella* detected belonged to *L.* nonpneumophila. In addition, there are also data of *Legionella* in drinking water reported to ILT where the proportion *L.* nonpneumophila with 56 to 76% is also higher than the proportion of *L. pneumophila* (Versteegh *et al.* , 2007). In the BEL study, several possible sources that patients with legionella pneumonia have been in contact with were investigated (Den Boer *et al.* , 2016). In this sampling, positive culture results were also split between *L. pneumophila* and *L.* nonpneumophila (Figure 7). When all samples were taken, it was also found that *L.* nonpneumophila was found more frequently than *L. pneumophila*, but the different source types did show different results. For example, In priority settings (sauna, hospitals, hotel), it was observed that *L. pneumophila* was observed more often than *L.* nonpneumophila, while in homes (non-priority setting), *L.* nonpneumophila was observed more often than *L. pneumophila*. However, it is important to add the nuance here that the BEL study examines source types that a patient has been in contact with, which in most cases has been an infection with *L. pneumophila*. Only in the study by Van der Kooij *et al.* (2007) were the *L.* nonpneumophila also characterized down to the species level.

In that 2007 study, 11,541 water samples from tap water systems in the Netherlands were analyzed for culturable Legionella. Culturable *Legionella* was found in 2,139 samples (18.5%) and further characterization showed that in 361 samples (3.1% of the number of samples tested) it was *L. pneumophila*, in 1,551 samples (13.4%) it was *L. anisa* and in the remaining 227 samples (2.0%) it was another Legionella species (Van der Kooij *et al.* , 2007).

These studies show that in Dutch drinking water *L.* nonpneumophila is found more frequently than *L. pneumophila* and that *L. anisa* is the most commonly found legionella species of the 21 legionella species described in the Dutch regulations. Despite the fact that *L. anisa* is considered the most dominant culturable legionella species in water in the Netherlands from tap water systems, the number of reported disease cases by *L. anisa* in the Netherlands is very low (with two reported cases in the last five years (Reukers *et al.* , 2020). This therefore means that there is a clear discrepancy between the presence of *L. anisa* in drinking water and the number of disease cases found, which is explained by the fact that *L. anisa* has a low virulence has and is hardly pathogenic (Fields *et al.* , 1990) and due to possible underdiagnosis.

In addition to pathogenic legionella species, research has shown that other opportunistic pathogens *(Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Aspergillus fumigatus,* non-tuberculous mycobacteria (NTM), *Waddlia chondrophila*) may be present in Dutch drinking water (Engel *et al.* , 1980, Van der Wielen & van der Kooij, 2013, Van der Wielen *et al.* , 2014, Van Dooremalen *et al.* , 2020). The disease caused by these microorganisms are partly nosocomial (hospital infection) and partly non-nosocomial and can lead to (small) outbreaks. In the Netherlands, for example, there was a small outbreak of *S. maltophilia* in 1996 in a hospital in which five premature babies became ill. In four of the five babies the infection was superficial, but the fifth baby died from the infection. The source of the infection appeared to be be the hospital's tap water system (Verweij *et al.* , 1998). *P. aeruginosa, S. maltophilia, A. fumigatus*, pathogenic NTM species and *W. chondrophila* can cause pneumonia just like *Legionella*, but depending on the organism they additionally cause eye infections, ear infections, skin infections, wound infections or infections of organs other than the lungs.

These microorganisms, like pathogenic *L.* nonpneumophila species opportunistically pathogenic, meaning that they primarily infect people with severely weakened immune systems.

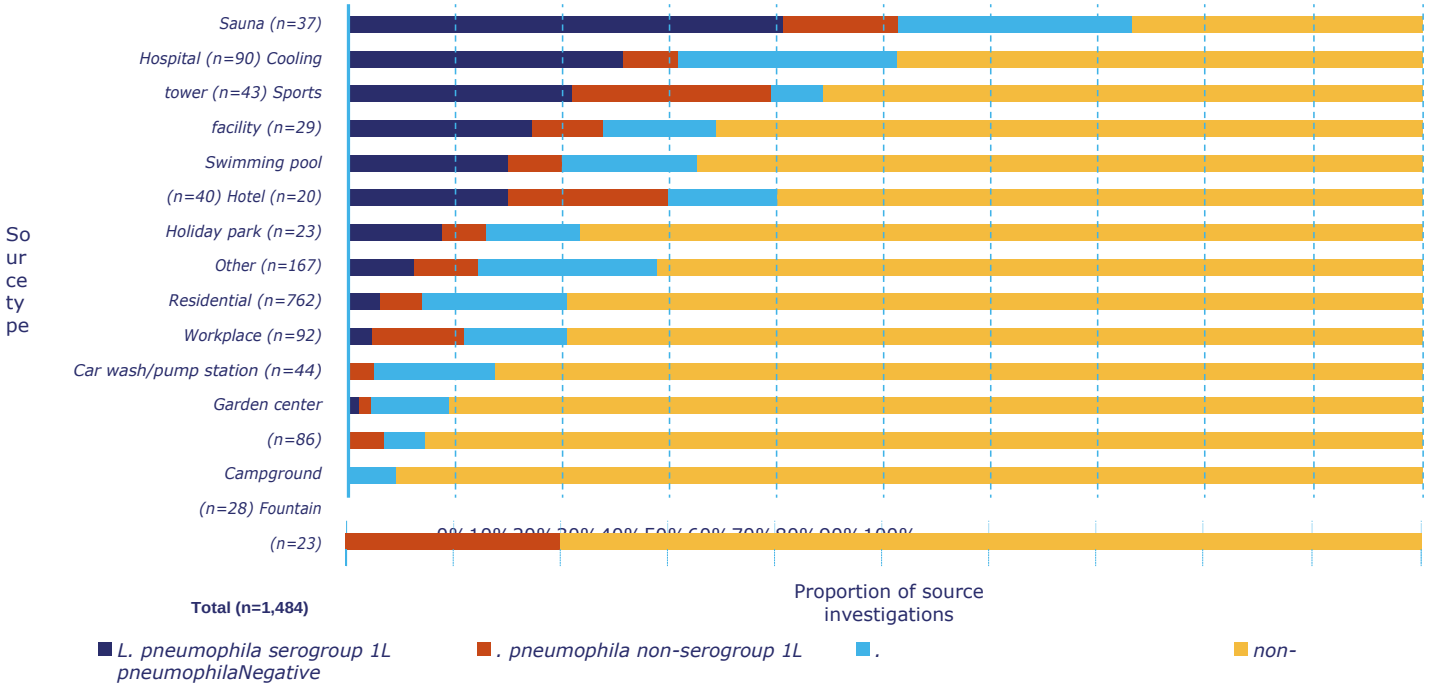


Figure 7. **Sampling results from BEL surveys over the period from 2002-2012 of 1484 potential sources of *Legionella* infection by source type. Source: Den Boer *et al.* (2016).**

In most countries infections caused by these other opportunistic pathogens are not notifiable, making it difficult to compare the number of reported cases with the number of cases caused by Legionella. Very recently, however, a study was conducted in the United States which used modeling to compare the number of estimated infections of seventeen waterborne pathogens. The results showed that the number of estimated reported cases of illness caused by NTM or *P. aeruginosa* (pneumonia and sepsis) is higher by a factor of five or six than the number of estimated reported cases of illness caused by *Legionella* (Collier *et al.* , 2021). This study does not include infections caused by *S. maltophilia* and *A. fumigatus* and does not distinguish between illnesses caused by *L. pneumophila* or *L.* nonpneumophila. It is also important to emphasize that this study included all water-related routes, so in addition to drinking water, for example, swimming pool water, natural swimming water, cooling tower water. So to what extent drinking water was responsible for these disease cases is not clear from the study. Although this American study is based on a large number of assumptions in the modeling, the results do show that disease cases caused by water-related NTM and *P. aeruginosa* also seems to be a serious problem in the United States.

Infections caused by other opportunistic pathogens are also not notifiable in the Netherlands. Because of this, it is unclear how many cases of disease there are in the Netherlands each year, but estimates have also been made based on epidemiology of reported cases.

The number of reported cases of NTM in the Nijmegen/Arnhem region was about 50 in 2005 (van Ingen *et al.* , 2009). At that time, 700,000 people lived in that region and 16.3 million in the Netherlands, which after linear extrapolation yields 1,164 cases of disease from NTM, but this assumes that the incidence in the Arnhem/Nijmegen region applies to the whole of the Netherlands. Also recently, a publication has estimated the current European incidence of NTM at 6.9 cases per 100,000 population (Schildkraut *et al.* , 2020). Extrapolating this figure to the Dutch situation results in 1073 cases of disease due to NTM in the Netherlands per year. An estimate of the number of patients who become ill from *A. fumigatus* in the Netherlands annually has also recently been calculated based on available epidemiological data (Buil *et al.* , 2020).
The results of this calculation showed that the number of estimated cases of disease caused by *A. fumigatus* per year in the Netherlands is 15,108, of which 1,283 cases with invasive aspergillosis and 257

cases with chronic pulmonary
aspergillosis, which can be life-threatening diseases.

Studies on incidence of disease cases with *P. aeruginosa* in the Netherlands was not found in publications, but in 2018, 13,151 clinical isolates of *P. aeruginosa* obtained (de Greeff *et al.* , 2019). This makes it plausible that the number of disease cases per year is also relatively high, although it remains unclear how many of these isolates actually caused disease. Although clear figures on the number of disease cases per year of these opportunistic microorganisms are lacking, because these diseases are not notifiable in the Netherlands, show published data and estimates clearly show that the number of annual disease cases of *P. aeruginosa*, NTM and *A. fumigatus* in the Netherlands is many times higher than the number of annual disease cases of *L.* nonpneumophila.

It is important to emphasize, however, that it is unknown how many of these disease cases are caused by drinking water, as these organisms also occur in many other sources (van der Wielen *et al.* , 2014), but the same is true for pathogenic legionella species. Incidentally, for *P. aeruginosa* and *A. fumigatus* established that the genotype of drinking water strains can be the same as the genotype of patient strains, showing that the drinking water strains of these microorganisms can also cause disease in the Netherlands (Van der Wielen & Wullings, 2019).

So although cases of disease with these other opportunistic pathogens are found much more frequently in the Netherlands than pathogenic *L.* nonpneumophila species, there is no legislation for this group of pathogens in drinking water. It is also known from research that the legal control measures that apply to *Legionella* do not in all cases also prevent the growth of these other pathogens. will control opportunistic pathogens in tap water systems. For example, it has been observed that *P. aeruginosa* can already multiply in drinking water biofilms when the water temperature is 15°C (Van der Wielen, 2020).

In summary, the following aspects emerge from the scientific literature:

- It is highly plausible that the number of cases of legionellapneunomy in the Netherlands is underreported due to limitations in diagnostics. Most patients are diagnosed with the urine antigen test in hospital, which reliably detects only *L. pneumophila* serogroup 1.
- When the cases of disease detected by culture are analyzed, it appears that, in particular, legionella pneumonia caused by *L. pneumophila* serogroup 2-14 and L. longbeachae is missed when relying only on urine antigen test. Legionella pneumonia cases caused by *L.* nonpneumophila species are also detected with culture only very sporadically.
- Unlike *L. pneumophila*, most pathogenic *L.* nonpneumophila species cause primarily infections in people with severely impaired immune systems.
- In Dutch drinking water, besides *L. pneumophila,* mainly *L. anisa* is detected. In the Netherlands almost no cases of disease caused by *L. anisa* have been described. The number of diagnosed and reported cases of disease caused by *L. anisa* is therefore lower than one per year. It is unclear, however, how large the underdiagnosis is for *L. anisa.*
- In addition to pathogenic legionella species, other pathogenic microorganisms have been found in Dutch drinking water that multiply in the tap water system and are not subject to drinking water regulations. The disease caused by these other pathogens are not notifiable, but estimates show that the number of disease cases is probably higher than the number of disease cases caused by pathogenic *L.* nonpneumophila species. For these other pathogens, as with pathogenic legionella species (including *L. pneumophila*), drinking water is not the only source of infection.
- Also, research has shown that control measures that apply to culturable *Legionella* do not all work against these other pathogens. For example, *P. aeruginosa* is able to multiply in drinking water biofilms at temperatures below 20°C.

In conclusion, based on current scientific knowledge, (i) it is highly plausible that targeting regulation in drinking water to the group of pathogenic *L.* nonpneumophila species will have little public health benefit and (ii) that targeting regulation to pathogenic *L.* nonpneumophila species is not in line with the absence of regulation of other opportunistic pathogenic microorganisms found in Dutch drinking water and of which there are probably more cases of disease in the Netherlands.

7.4.3 Masking of *L. pneumophila* by *L.* nonpneumophila

The method prescribed by law to determine *Legionella* spp in drinking water samples is described in ISO 11731 and describes culture in which the BCYE agar medium serves as a base and to which antibiotics are added to achieve selectivity for growth of *Legionella*. This selective agar medium was originally developed for clinical detection of *L. pneumophila* (Lee *et al.* , 1993). As previously described, an extensive study of growth of eighteen different legionella species on the liquid BCYE medium (i.e., without the addition of agar) show that *L. pneumophila* is able to multiply better in the BCYE medium than the seventeen *L.* nonpneumophila species. Growth of *L. birminghamensis, L. cherrii, L. cincinnatiensis, L. dumoffli, L. Iongbeachae, L. santicrucis, L. steigenvaltii* were thereby marginal compared to growth of the other fourteen legionella species, resulting in colonies of small diameter (1 mm) compared to colonies of *L. pneumophila* (3 to 5 mm). Four of these worse growing legionella species are also mentioned in the Dutch legislation and it is not inconceivable that due to the small colony size of these species, these colonies are not counted as typical legionella colonies by laboratories. From the 21 legionella species listed in the law are *L. lansingensis* and *L. waltersii* were not included in the study by Van Lee *et al.* (1993), but these organisms were cultured from patients using the BCYE agar medium (Thacker *et al.* , 1992, Benson *et al.* , 1996) and thus also capable of growing on the BCYE agar medium.

Because both *L. pneumophila* and other legionella species are capable of growing on the culture medium, it is possible that the presence of *L. pneumophila* in a water sample could be masked by other culturable legionella species if they are present in higher numbers than *L. pneumophila*.

Studies in pilot plants that were fed with Dutch drinking water have shown that at water temperatures between 30 and 38.5°C *L. anisa* and *L. pneumophila* can occur together, where at the lower temperatures (30 to 34°C) it was observed that the numbers of *L. anisa* were significantly higher than those of the respective strain of *L. pneumophila* (Van der Kooij *et al.* , 2009). In such situations, determination of culturable *Legionella* according to ISO 11731 would lead to detection of *L.* nonpneumophila, while *L. pneumophila* is also present in the water samples. Several foreign studies also report that both *L. pneumophila* and *L.* nonpneumophila can be found in water samples, including samples taken from building tap water systems (e.g. Cassier *et al.* , 2013, Cassini *et al.* , 2017, Dilger *et al.* , 2018, Johnson *et al.* , 2018). Thus, this shows that multiple culturable legionella species can be present in the same plant and/or drinking water samples.

In the Netherlands, some of the patients with Legionella pneumonia are examined to see if the same strain can also be found in known sources for *Legionella* and to which the person was exposed during the incubation period (the so-called source detection unit *Legionella* (BEL) examination). A systematic record of all BEL examinations is lacking and publications are lacking from The BEL survey in which data on presence of *L. pneumophila* and *L. anisa* is found in the same tap water system. However, the BEL study investigators reported via email that *L. pneumophila* and *L.* nonpneumophila have been found together several times in a tap water system.

Some other studies in the Netherlands have also looked at the presence of *L. pneumophila* and *L.* nonpneumophila in drinking water samples from piping systems (Van der Kooij *et al.* , 2007, Van der Lugt *et al.* , 2019). Often, these studies report the percentage of positive samples for *L. pneumophila* and *L.* nonpneumophila, but do not indicate whether both were found in the same plant. In another study where water samples from Dutch tap water systems were analyzed using culture methods for *Legionella*, *L. anisa* and *L. pneumophila were* both found in three of 107 samples (Veenendaal *et al.* , 2017).

Because these studies did not include a specific detection method for
L. pneumophila was applied, it remains unclear whether in the other samples where *L. anisa* or *L.* nonpneumophila was found, *L. pneumophila* was also present in lower numbers. Samples taken as part of the BEL study also show examples where *L. pneumophila* and *L.* nonpneumophila were both found in one tap water system (information from RIVM). It is clear that *L. pneumophila* and *L.* nonpneumophila can also be present together in tap water systems in the Netherlands.

To find out if *L. pneumophila* is present in lower numbers in drinking water samples when *L.* nonpneumophila is found, water samples should be analyzed using the ISO 11731 culture method and a method that specifically detects *L. pneumophila*. Several companies claim to have developed specific methods for detection of *L. pneumophila*, but often lack
a scientific validation (e.g., according to ISO 17994) of such methods making it unclear
is how reliable such methods can detect *L. pneumophila* in drinking water samples. Some methods that specifically detect *L. pneumophila* do have scientific articles and/or a standardized method exists. For example, a quantitative PCR (qPCR) method has been developed for *L. pneumophila*, the analytical method of which is covered by ISO 12869. Nevertheless, a comparison between this qPCR method and the culture method to find out whether culturable *L. pneumophila* is present when *L.* nonpneumophila is detected is less appropriate, because the qPCR method can detect nonculturable and dead *L. pneumophila* in addition to culturable ones.
However, in addition to the qPCR method, a culture method using a selective agar medium and incubation temperature for *L. pneumophila* has also been described and limited validated with drinking water samples from Dutch piping systems
(Veenendaal *et al.* , 2017) as well as a culture method using a selective liquid medium and color conversion (Legiolert™/ Quanti-Tray®) that has been extensively validated with drinking water from pipeline systems in Germany, United States and Italy (Sartory *et al.* , 2017, Petrisek & Hall, 2018, Spies *et al.* , 2018, Scaturro *et al.* , 2020).

A selective culture method based on an agar medium showed that, in addition to *L. pneumophila*, only *L. adelaidensis* and *L. londiniensis* grow under the selective breeding conditions (Veenendaal *et al.* , 2017), but these two *L.* nonpneumophila species are not pathogenic and therefore not explicitly included in the regulations (Brandsema & Schalk, 2010). The remaining 24 tested *L.* nonpneumophila species were not able to reproduce. The results of the analyses of field samples from Dutch tap water systems showed that 26 samples were positive for *L. anisa* and 20 samples for another *L.* nonpneumophila species. However, in these 46 samples the culture for *L. pneumophila* was negative (Veenendaal & Van der Kooij, 2008, Veenendaal *et al.* , 2017). Thus, in
These 107 samples were not found to mask the presence of *L.* nonpneumophila. Validation studies of the Legiolert™/Quanti- Tray® did report the presence of *L.* nonpneumophila in some drinking water samples from piping systems.

However, these samples were not included in the further validation of the methods, so it remains unclear to what extent *L.* nonpneumophilapositive samples were also positive with the selective culture method for *L. pneumophila (*Sartory *et al.* , 2017, Petrisek & Hall, 2018, Spies *et al.* , 2018,
Scaturro *et al.* , 2020). The general picture from these studies, however, was that with the Legiolert™/Quanti-Tray® as many or more samples were positive for *L. pneumophila* than the culture method according to ISO 11731 and that also the numbers of
L. pneumophila were generally higher with Legiolert™/ Quanti-Tray® than with the traditional culture method. This seems to indicate that the agar culture method according to ISO 11731 has a lower yield of *L. pneumophila* than Legiolert™/ Quanti-Tray®. Also, the specificity of the Legiolert™/ Quanti-Tray® was between 96.7 and 100% (Sartory *et al.* , 2017, Petrisek & Hall, 2018, Spies *et al.* , 2018), which is higher than the 95.3% specificity for the ISO 11731, as reported in the latest version of this ISO protocol.

From the review of the scientific literature, pilot plant studies indicate that there are conditions under which the presence of *L.* nonpneumophila can
of *L. pneumophila* can mask when the traditional culture method according to ISO 11731 is applied. It has also been shown that *L. pneumophila* and *L.* nonpneumophila can occur together in detectable numbers in a plant or drinking water samples.

To what extent the presence of *L. pneumophila* in field samples is masked by *L.* nonpneumophila, when analyses are employed according to ISO 11731, cannot be indicated from the scientific literature due to the limited amount of data. Meanwhile, at least two promising alternative culture methods for the specific detection of *L. pneumophila* in drinking water samples from piping systems have been published. The application of those culture methods makes it possible to to be able to focus detection exclusively on *L. pneumophila*, without *L.* nonpneumophila interfering with the detection of *L. pneumophila*. However, it is important that these methods are standardized and normalized according to the methodologies of the national standards organization (NEN) or the international standards organization (ISO). In this regard, the Legiolert™/Quanti-Tray® does already have a standardization protocol according to the French standardization organization (AFNOR) and is also included in the "Blue Book" of validated test methods in the United Kingdom. Also, the validation studies have shown that under-reporting of the number of *L. pneumophilapositive* samples and the numbers of *L. pneumophila* in drinking water samples from piping systems when the ISO 11731 culture method is used instead of Legiolert™/Quanti-Tray®.

7.4.4 Culturable *L.* nonpneumophila as an indicator organism for *L. pneumophila*

From a practical point of view, a number of people have indicated that finding *L.* nonpneumophila in drinking water and/or hot tap water is an indication that there are problems with the management of the plant, which ultimately *L. pneumophila* would be able to reproduce in the plant. However, scientific support for this position is lacking. If *L.* nonpneumophila is an indication that management of the plant is not sufficient, then these *L.* nonpneumophila species should only be multiply in the installation when the measures currently in force from legionella legislation are not complied with. For example, these *L.* nonpneumophila species should not propagate if the drinking water temperature is below 25°C or above 55 to 60°C, measures that are directly linked to propagation of *L. pneumophila*. So, in effect, this means that propagation of *L.* nonpneumophila is used as an indicator organism for propagation of *L. pneumophila*. Therefore, this section finds out whether there is a scientific basis for the use of *L.* nonpneumophila as an indicator of *L. pneumophila*.

Indicator organisms have been used for decades to monitor drinking water quality, and particularly to identify the health risk posed by the presence of fecal pathogens in drinking water in a timely manner. In the scientific literature, several publications have therefore been published describing what an indicator organism should meet (Bonde, 1966, Council, 2004, Yates, 2007, Dufour *et al.* , 2013). The publication by Dufour *et al.* (2013) is a World Health Organization publication. The main criteria invariably cited for an indicator organism are:

1. the indicator organism must always be present when the pathogen is present
2. the indicator organism must be present in higher numbers than the pathogen
3. the ecology of the indicator organism must be the same as the pathogen
4. The indicator organism must be more resistant to disinfection than the pathogen.
5. growth of the indicator organism on the selective culture medium is independent of growth of other microorganisms on the culture medium.

Based on these five criteria, the extent to which culturable *Legionella* spp appears to be a good indicator organism for *L. pneumophila* is examined.

1st criterion: the indicator organism must always be present when the pathogen is present According to this first criterion, culturable *L.* nonpneumophila species are always present when *L. pneumophila* is found. However, it is difficult to determine whether this is true in all cases because *L. pneumophila* also grows on the culture medium used to determine *L.* nonpneumophila. For example, a number of studies report that only *L. pneumophila* was detected using the traditional culture method according to ISO 11731. However, in those studies, *L.* nonpneumophila may also be present in the water samples analyzed, but in lower numbers than *L. pneumophila*.

Studies in which specific culture methods for *L. pneumophila* were tested against the traditional culture method for *Legionella* spp show that drinking water samples can be positive with the specific culture method for *L. pneumophila*, but negative with the specific culture method for *Legionella* spp (Veenendaal *et al.* , 2017, Sartory *et al.* , 2017, Petrisek & Hall, 2018, Spies *et al.* , 2018, Scaturro *et al.* , 2020). Thus, in those cases, the traditional culture method for *Legionella* spp was negative, including growth of *L. pneumophila*, which is remarkable because the other specific method did show that *L. pneumophila was* present in the sample. Veenendaal *et al.* (2017) showed that this was caused by (i) the numbers of *L. pneumophila* using the specific culture method being just above the detection limit, so it is possible that it was just below the detection limit for the traditional culture method and (ii) interference flora on the traditional culture medium for *Legionella* spp was such that the detection limit for culturable *Legionella* spp was significantly higher than for culturable *L. pneumophila* where interfering flora did not interfere with the specific culture method. Thus, in those cases, it was not possible to detect *L.* nonpneumophila even though *L. pneumophila* was present.

In a study where drinking water samples from tap water installations in Germany were analyzed using the Legiolert™/Quanti-Tray® method, as a specific method for the detection of *L. pneumophila*, and using traditional culture according to ISO 11731, only the Legiolert™/ Quanti-Tray® method proved positive in 5.8 to 10.0% of the positive samples for *L. pneumophila* (Spies *et al.* , 2018). In 17 of these samples, the numbers found using Legiolert™/Quanti- Tray® were higher than the German standard of 1,000 cfu/l. Thus, in these cases *L. pneumophila* was also found while culturable *Legionella* spp were not present. It follows from this inventory that not many publications were found in the scientific literature that attempted to find out whether culturable *Legionella* spp are present when *L. pneumophila* is found.

Also, with the current traditional culture method for *Legionella* spp, this is also difficult to ascertain, because both the indicator organism (*Legionella* spp) and the pathogen (*L. pneumophila*) grow on the culture medium used. The few studies comparing the culture of *L. pneumophila* performed by a different culture method with the traditional culture method showed that mostly culturable *L.* nonpneumophila were not found when *L. pneumophila* was detected, but it may be that they were present in lower numbers than culturable *L. pneumophila*. In addition, it was seen that in 5 to 10% of the samples L. nonpneumophila was not present was, while *L. pneumophila* was detected. It can be concluded from this that culturable *L.* nonpneumophila as an indicator organism does not meet the criterion that it is always present when the pathogen is present.

2nd criterion: the indicator organism must be present in higher numbers than the pathogen

Thus, in the case that culturable *L.* nonpneumophila is used as an indicator of *L. pneumophila*, this would mean that the numbers of *L.* nonpneumophila should always be higher than *L. pneumophila*. Many studies have used the numbers of *L. pneumophila* and *L.* nonpneumophila in drinking water samples from field plants examined by the traditional culture method (Ezzeddine *et al.* , 1989, Darelid *et al.* , 2002, Borella *et al.* , 2005, Leoni *et al.* , 2005, Moore *et al.* , 2006, Mouchtouri *et al.* , 2007, Stout *et al.* , 2007, Versteegh *et al.* , 2007, Hrubá, 2009, Arvand *et al.* , 2011, Arvand & Hack, 2013, Barna *et al.* , 2016, Kruse *et al.* , 2016, Collins *et al.* , 2017, Dilger *et al.* , 2018).

In doing so, the results of most studies show that regularly only *L. pneumophila* is cultured, but not L. nonpneumophila species. As explained earlier, it is possible that *L.* nonpneumophila is present but not is detected because the numbers are lower than those of *L. pneumophila*. Culturable *L.* nonpneumophila therefore does not meet this second criterion of an indicator organism. In itself, this is also logical, since the applied traditional culture method is particularly developed to identify the pathogen (*L. pneumophila*) and not as a culture method to detect indicator organism for *L. pneumophila*. It shows again that one and the same culture method for both indicator organism and pathogen is not desirable and hinders reliable application as indicator organism.

3rd criterion: The ecology of the indicator organism should be the same as the pathogen

More than sixty different species belonging to the genus *Legionella* have been described today, many of which can reproduce on traditional culture medium according to ISO 11731 (National Academies of Sciences, 2019). However, knowledge of the ecology of many of these species is very limited. Because in Dutch drinking water much- al *L. anisa* is found as *L.* nonpneumophila species (Van der Kooij *et al.* , 2007, Versteegh *et al.* , 2007) we limit the comparison of ecology to that of *L. anisa* and *L.pneumophila*.

The ecology of *L. pneumophila* in drinking water systems is characteristic in that the organism multiplies in host protozoa that graze on biofilm (Kuijper *et al.* , 1989, National Academies of Sciences, 2019). Laboratory studies have shown that *L. anisa* can also replicate in protozoa (Fields *et al.* , 1990, Steele & McLennan, 1996, La Scola *et al.* , 2001), although it has been reported that unlike *L. pneumophila, L. anisa* could not be observed in the vacuoles of the protozoa (Storey *et al.* , 2004). However, studies on the extent to which *L. anisa* can multiply in drinking water systems with and without protozoa are lacking. In any case, little research has been conducted on the ecology of *L. anisa* in the drinking water ecosystem. Studies with Dutch drinking water have shown that growth of *L. anisa* is stimulated by the addition of iron rust particles (Van der Lugt *et al.* , 2017), which is similar to the observations that increased numbers of *L. pneumophila* are found in sites with elevated iron concentrations (Fisher-Hoch *et al.* , 1982, States *et al.* , 1985, Van der Kooij *et al.* , 2020).

Another study with *L. anisa* and *L. pneumophila* in a pilot piped water system fed with Dutch drinking water showed that the temperature range at which *L. anisa* and *L. pneumophila are* able to reproduce differ from each other (Van der Kooij *et al.* , 2009). The results showed that *L. anisa* is better able to reproduce at lower drinking water temperatures than *L. pneumophila* and that *L. pneumophila* is better able to reproduce at higher drinking water temperatures. As a result, in the study by Van der Kooij *et al.* (2009), at the lower temperatures (mostly < 30°C) only *L. anisa* was found in the biofilm, while at the higher temperatures (> 38°C) only *L. pneumophila* was found in the biofilm. A study examining the influence of temperature on growth of *L. pneumophila* in host protozoa confirmed this picture, as propagation of *L. pneumophila* in host protozoa did not take place at 24°C, but at 30°C and above (Buse & Ashbolt, 2011).

Field studies in which drinking water samples from tap water systems were analyzed for culturable *Legionella* spp also confirm the view that drinking water temperature has a different effect on growth of L. nonpneumophila and *L. pneumophila*. In many of these studies, it is found that at lower drinking water temperatures (e.g., samples from cold tap water portion of the plant), mainly *L.* nonpneumophila is found (Oesterholt & Veenendaal, 2002, Mouchtouri *et al.* , 2007, Van der Kooij *et al.* , 2007, Van Hoof *et al.* , 2014, Van der Lugt *et al.* , 2017, Van der Lugt *et al.* , 2019), while at higher drinking water temperatures (e.g. samples from hot tap water part of the plant) mainly *L. pneumophila* is found (Ezzeddine *et al.* , 1989, Borella *et al.* , 2005, Leoni *et al.* , 2005, Moore *et al.* , 2006, Mouchtouri *et al.* , 2007, Stout *et al.* , 2007, Barna *et al.* , 2016, Kruse *et al.* , 2016, Dilger *et al.* , 2018). Nevertheless, there are also studies in which *Legionella* nonpneumophila is found more frequently than *L. pneumophila* in warm water, but identification to the species level of *L.* nonpneumophila found was not performed in those studies (Darelid *et al.* , 2002). Based on the data on the influence of temperature on growth and detection of *L. pneumophila* and *L. anisa*, it is concluded that the ecology of *L. pneumophila* and *L. anisa* regarding growth temperature is partly different from each other.

Other important ecological conditions that promote the growth of *L. pneumophila* in drinking water systems affect are nutrient concentrations, water quality, and piping materials (Van der Kooij, 2014, National Academies of Sciences, 2019). However, studies of these ecological conditions on growth of L. nonpneumophila species have not been found, so it is not possible to make a statement on the extent to which the ecology of *L. pneumophila* and *L.* nonpneumophila are similar with respect to these conditions.

From the scientific publications, it can be concluded that the ecology between the indicator organism (*L.* nonpneumophila) and the pathogen (*L. pneumophila*) is not the same for all major environmental conditions. At least part of the temperature range at which growth occurs appears to be different between *L. anisa* and *L. pneumophila*, so the presence of *L. anisa is* not a good indicator of *L. pneumophila* under all conditions. As a result, *L. pneumophila* may be present at higher temperatures while *L. anisa* is absent, and *L. anisa* may be present at lower temperatures while *L. pneumophila* is not able to grow at those temperatures. Thus, the indicator organism does not comply with this third criterion.

4th criterion: the indicator organism must be more resistant to disinfection than the pathogen

Not many laboratory-controlled studies have been conducted on the influence of different disinfection methods on species of *L.* nonpneumophila. In one of the few scientific studies found, the influence of thermal disinfection or chlorine on protozoal *L. pneumophila* and *L. erythra* compared (Storey *et al.* , 2004). The results showed that no significant differences were observed between the effect of the disinfection methods on *L. pneumophila* and *L. erythra*. More studies were found in which real-world disinfection methods were isolated and monitored for effectiveness against the *L. pneumophila* and *L.* nonpneumophila. Results from some of those studies showed, for example, that copper silver ionization, thermal management, hydrogen peroxide with silver ions, and hyperchlorination killed off both *L. pneumophila* and L. nonpneumophila (*L. anisa* and/or *L. rubrilucens*) present to below the detection limit in tap water systems of health care facilities (Orsi *et al.* , 2014, Dziewulski *et al.* , 2015, Girolamini *et al.* , 2019, Lecointe *et al.* , 2019). However, multiple studies have shown that *L. anisa* or *L.* nonpneumophila are more sensitive to thermal disinfection than *L. pneumophila* (Kruse *et al.* , 2016; Mouchtouri *et al.* , 2007, van der Mee-Marquet *et al.* , 2006). Whereas, a study using only hydrogen peroxide showed that it was effective in killing off *L. pneumophila*, but that certain *L.* nonpneumophila species were less susceptible (Casini *et al.* , 2017). Although not very many scientific studies are available under which controlled conditions the influence of disinfection methods on *L. pneumophila* and *L.* nonpneumophila has been studied, field studies appear to show that *L. pneumophila* and *L.* nonpneumophila present in tap water systems generally respond similarly to disinfection methods, except for thermal disinfection. *L.* nonpneumophila, as indicator organisms for *L. pneumophila*, thus partially satisfies this fourth criterion.

5th criterion: growth of the indicator organism on culture medium is independent of growth of other microorganisms on the culture medium

In addition to these four biological criteria defined for an indicator organism, criteria were also defined for the methodological aspects for detecting the indicator organism (Yates, 2007). An important criterion here is that growth of the indicator organism on a culture medium is independent of growth of other microorganisms (Bonde, 1966, Yates, 2007). Because *L.* nonpneumophila and *L. pneumophila* are determined on the same culture medium, this criterion is not met because when culturable *L. pneumophila* is present in higher numbers than *L.* nonpneumophila in a water sample, it is not clear whether the indicator organism (*L.* nonpneumophila) is also present. As explained earlier, this aspect also makes it very difficult to determine if *L.* nonpneumophila is always present when *L. pneumophila* is encountered. The use of *L.* nonpneumophila as an indicator organism for *L. pneumophila* therefore does not meet this fifth criterion.

This analysis shows that *L.* nonpneumophila as an indicator organism for the pathogen *L. pneumophila* does not meet four of the five criteria and partially meets one of the five criteria set for an ideal indicator organism by international scientific studies, including those of the WHO. Incidentally, it is true that almost all indicator organisms currently in use do not meet all of these criteria. However, *L.* nonpneumophila only partially meets one of the five criteria, which is very little to make *L.* nonpneumophila reliable as an indicator organism for *L. pneumophila*. This is also the most likely re- den that none of the recent reviews of indicator organisms for fecal contamination and aftergrowth in drinking water systems mention *Legio nella* spp as a possible indicator for *L. pneumophila* (Council, 2004, Yates, 2007, Dufour *et al.* , 2013).

In 2006, however, a scientific publication was published with the title: '*Legionella* anisa, a possible indicator of water contami- nation by *Legionella* pneumophila' (van der Mee-Marquet *et al.* , 2006), which suggests that *L. anisa* can be used as an indicator organism for *L. pneumophila* after all. However, the study by van der Mee-Marquet *et al.* (2006) does not examine the extent to which *L. anisa* can be used as an indicator organism for *L. pneumophila*, but rather examined the extent to which the detection of *L. anisa* by the selective culture method according to ISO 11731 can mask the presence of *L. pneumophila*. In certain water samples taken from the tap water system after heat shock treatment, ISO 11731 detected *L. anisa* but not *L. pneumophila*.

However, a qPCR method specific to *L. pneumophila* showed that DNA of *L. pneumophila* was present in these samples. Thus, in those samples, the presence of culturable *L. anisa* possibly masked the presence of culturable *L. pneumophila* (as also described in Section 7.3.3), although it remains unclear to what extent the *L. pneumophila* DNA found was from live *L. pneumophila*. The limitation of growing both the indicator organism *L. anisa* and the pathogen *L. pneumophila* on the same medium also prevents the reliable detection of live *L. pneumophila*. For the practical situation, the scientific finding means that the ecology of culturable *L.* nonpneumophila species is not the same as that of *L. pneumophila* and that *L.* nonpneumophila can be found in an installation even though the control measures taken comply with current legionella legislation. In addition, thermal disinfection can successfully *L.* nonpneumophila control, while *L. pneumophila* is not killed or is killed less by this control measure. Thus, based on the scientific knowledge, it can be concluded that *L.* nonpneumophila is not a good indicator organism for *L. pneumophila* and that the detection of *L.* nonpneumophila is an unreliable parameter for determining whether the management of a facility against *L. pneumophila is* in order.

7.4.5 Effectiveness of control measures against different legionella species

In essence, the cornerstones of the control of *Legionella* in tap water installations according to the Dutch Regulation on Legionella prevention in drinking water and hot tap water are that the cold tap water has a drinking water temperature lower than 25°C, the hot tap water has a temperature of 55°C or higher and the water from the hot tap water device has a temperature of 60°C or higher. However, in addition to thermal control measures, physical control measures may also be applied. Should these control measures not sufficiently lead to control of culturable *Legionella* spp, then electrochemical control measures may also be applied (with a necessary substantiation from a certified agency).

Under the fourth criterion in the previous section, the scientific knowledge about the effect of disinfection methods, including thermal and chemical management methods, has already been discussed. From the analysis it was concluded that not many studies have examined the extent to which *L.* nonpneumophila species react in the same way as *L. pneumophila* on the various control measures. Nevertheless, scientific literature has shown that a hot water temperature of 55°C in the water

sampled from distal taps of an installation and a hot tap water temperature of 60°C at the hot tap water heater ensures control of all culturable *Legionella* spp (see Chapter 3). Based on this, it can be concluded that one of the two key points regarding Legionella management in the Dutch regulations is effective against both *L. pneumophila* as *L.* nonpneumophila species.

The influence of cold water temperature on growth of *L. pneumophila* and *L.* nonpneumophila was discussed at several locations in the report. The most important Observation in this regard is that *L. anisa* seems to be able to reproduce at lower drinking water temperatures than *L. pneumophila*. Using a qPCR method that detects all legionella bacteria (culturable legionella species and (as yet) non-culturable legionella species), it was found that especially non-culturable legionella bacteria are present in high numbers in drinking water and other water types at temperatures below 20°C (Wullings & van der Kooij, 2006, Carvalho *et al.* , 2008, Parthuisot *et al.* , 2010, Wullings *et al.* , 2011). This shows that certain undescribed species of the genus *Legionella* are able to multiply at colder water temperatures.

Culturable species of *L.* nonpneumophila have also been observed in relatively high numbers using the culture method on BCYE agar at water temperatures between 20 and 25°C (Rogers *et al.* , 1994, Riffard *et al.* , 2001, Pryor *et al.* , 2004, Versteegh *et al.* , 2007, Arvand *et al.* , 2011, van der Lugt *et al.* , 2017, van der Lugt *et al.* , 2019). In some of these studies, the culturable *L.* nonpneumophila found were characterized to species level and this showed that the following legionella species were found: *L. anisa, L. dumoffi, L. erythra, L. fallonii , L. feelei,L. geestiana, L. gormanii,L. gresilensis, L. parisiensis, L. quateirensis, L. rubrilucens, L. santicrucis and L. waltersii* (Riffard *et al.* , 2001, Pryor *et al.* , 2004, Versteegh *et al.* , 2007, an der Kooij *et al.* , 2009, van der Lugt *et al.* , 2017, van der Lugt *et al.* , 2019).

Studies in which legionella populations have been characterized by molecular methods also show that some described legionella species (e.g., *L. anisa, L. parisiensis, L. maceachernii, L. birminghamensis, L. erythra, L. bozemanii, L. worsleiensis, L. quateirensis, L. waltersii, L donaldsonii, L. yabuuchiae, L. lytica)* may be present in drinking water samples whose temperature is below 25°C, but *L. pneumophila* was also detected in low numbers (Calvo-Bado *et al.* , 2003, Wullings & van der Kooij, 2006, Wullings *et al.* , 2011). The applied culture methods for

Legionella were otherwise negative in these three studies. Also, multiplication of *L. anisa* at temperatures between 20 and 25°C was demonstrated in a pilot tap water system fed with Dutch drinking water (Van der Lugt *et al.* , 2017, Van der Lugt *et al.* , 2019). Thus, the results of these studies show see that several L. nonpneumophila species can breed in installations with drinking water temperatures between 20 and 25°C. Based on these results, it can be concluded that one of the two key points regarding legionella management in the Dutch regulations is not effective in preventing growth of certain culturable *L.* nonpneumophila species.

Indeed, in order to prevent the growth of these cultivable *L.* nonpneumophila species, the cold water temperature in a tap water system should not exceed 20°C. However, this is unrealistic in practice because (i) in the summer period at drinking water companies using surface water as a source, the temperature of the raw material is already often exceeds 20°C, (ii) warming occurs during distribution of drinking water due to hotspots in urban areas, among other things, and (iii) drinking water warms during distribution in buildings. It also follows from these data that *L.* nonpneumophila will be found more frequently than *L. pneumophila* if the cold water temperature is below 25°C.

Thus, finding *L.* nonpneumophila is not a reliable indication of whether the management of the plant is in order against *L. pneumophila*, since *L.* nonpneumophila can be found while the drinking water temperature is between 20 and 25°C.

7.5 Conclusion scientific status of *Legionella* species

In this chapter, the scientific literature was surveyed to find out whether monitoring *L.* nonpneumophila in addition to *L. pneumophila* is meaningful because it poses a risk to public health, the presence of *L. pneumophila* can mask or serve as an indicator organism for *L. pneumophila*. Based on the scientific literature consulted, the following partial conclusions are drawn:

- It is plausible that targeting regulations in drinking water to the group of pathogenic *L.* nonpneumophila species little gain for the public health.
- Focusing on regulations regarding disease-causing *L.* nonpneumophila species is not in line with the absence of regulation of other opportunistic pathogenic microorganisms in drinking water that are more virulent and of which more cases of disease are observed in the Netherlands.
- It follows from pilot plant studies that conditions may be present under which the presence of *L.* nonpneumophila, can mask the presence of *L. pneumophila* when the traditional culture method according to ISO 11731 is applied. In addition, *L. pneumophila* and *L.* nonpneumophila are present together in a tap water system, making it plausible that also in field samples *L.* nonpneumophila can mask the presence of *L. pneumophila* when analyses are deployed according to ISO 11731.
- Culture methods for the specific detection of *L. pneumop hila* are available. When such methods are applied, the problem that L. nonpneumophila masks the presence of *L. pneumophila* no longer arises.
- *L.* nonpneumophila, as an indicator organism for the pathogen *L. pneumophila*, does not meet four of the five criteria set by international scientific studies, including those of WHO, for an ideal indicator organism. Thus, *L.* nonpneumophila cannot be used as a reliable indicator organism for *L. pneumophila*.
- Due to the difference in ecology of L. nonpneumophila and *L. pneumophila* and the difference in killing of *L.* nonpneumophila and *L. pneumophila* in thermal disinfection, *L.* nonpneumophila is an unreliable parameter for determining whether the management of a facility is effective against *L. pneumophila*.
- The management measure of ensuring that hot tap water is higher than 55°C and that water from the hot water heater is higher than 60°C, one of the two cornerstones of the Regulation on Legionella Prevention in Drinking Water and Hot Tap Water, is also effective against *L.* nonpneumophila species detected by the culture method according to ISO 11731. In contrast, the management measure of ensuring that cold tap water is below 25°C, the other cornerstone of the Regulations, is not effective against all *L.* nonpneumophila species that can be used with the culture method

according to ISO 11731 are detected.

- To ensure that the control measures listed in the regulations Legionella prevention in drinking water and hot tap water meet all legionella species listed in the regulations, the requirement for the cold water temperature should be lowered to 20°C, which is not realistic in practice.

Based on current scientific knowledge regarding pathogenic L. nonpneumophila and the use of *L.* nonpneumophila as an indicator for *L. pneumophila* or to identify facilities where management is up against *L. pneumophila* is insufficient, it is concluded that targeting legislation for most priority settings to all culturable legionella species rather than culturable *L. pneumophila* contributes little to preventing cases of legionella pneumonia, provided standardized specific detection methods for culturable *L. pneumophila* can be employed.

However, in locations where many people with severely weakened immune systems visit (e.g., hospitals, nursing homes), legislation targeting all culturable *Legionella* spp makes sense because these people are susceptible to pathogenic *L.* nonpneumophila species.

7.6 Knowledge and experiences from practice

A number of respondents indicated that they did not want to go straight for regulation with a focus on *L. pneumophila*. Arguments are the possible role of *L.* nonpneumophila as an indicator of the presence of *L. pneumophila* , the chance of missing other pathogenic Legionella species and a better quality awareness by the operator of a facility when detecting all culturable *Legionella* spp. However, the possibility is mentioned that in high-priority settings (think of health care institutions) to look at all culturable *Legionella* spp and at the other priority settings only *L. pneumophila*. Another respondent is in favor of maintaining the current standard but with different action levels and notification limits for *L.* nonpneumophila and *L. pneumophila*.
Practitioners need clarity on what actions should be taken when standards are exceeded depending on concentration and type of *Legionella*. Incidentally, that kind of detail would fit better in the ISSO 55.1 than in the regulations.

Respondents from the disability sector especially feel that they have to take many measures for legionella species from which only people with severe weakened immune system become ill. These generally do not live in their institutions. The industry's interest is primarily that the regulations can be organized in a way that is in the best interest of their residents. Currently, the regulations result in doing unnecessary work that costs a lot of time and money, but which in practice is not at all in the best interest of the residents. So that's separate from the technology and the question of whether effective legionella management can be carried out. Safety is always paramount in this regard, by the way, and the institutions are even willing to do extra things if necessary for the safety of the residents. But what particularly bothers the sector is that a lot of work has to be done, while the target group does not belong to a risk group. In many locations, the actual risk is therefore low and yet a lot of time and money must be spent on the work.
be invested and that is no longer explainable. The rules now de facto determine what is done about legionella prevention, while that is in fact the real risk for residents should be. A focus on *L. pneumophila* could help them in that regard. At one organization, 700 - 800 samples are taken annually. Exceedances of standards almost always involve *L.* nonpneumophila. Exceptionally *L. pneumophila* is found.

A team of 2.5 FTEs is now constantly implementing management measures. At another organization, more than 1,000 samples are taken annually. Outgoing
of 2,000 to 3,000 samples in recent years, they have had perhaps two instances of norm exceedance with *L. pneumophila*.

7.7 Recommendation to adjust regulations based on scientific insights

The conclusion of the scientific survey, namely that legislation for most priority settings should focus on culturable *L. pneumophila* in combination
with the application of a specific culture method for *L. pneumophila*, is not in line with the current regulations. The current regulations focus on culturable *Legionella* spp and for monitoring according to the management plan it is included that monitoring is performed on culturable *Legionella* spp according to ISO method 11731. If the legislation were to focus on *L. pneumophila*, it would be necessary to be able to specifically detect *L. pneumophila* in drinking water sample.

Therefore, there was also a limited examination of whether specific detection methods for *L. pneumophila* are described in the scientific literature. In particular, the Legiolert method
seems appropriate and in some countries has also been described as a standardized method, but an additional literature review and possibly field study of possible specific detection methods for *L. pneumophila* is needed before a recommendation can be made for such a method.

Current scientific knowledge also shows that some cultivable *L.* nonpneumophila species, especially the species *L. anisa,* which is dominant in drinking water, is able to reproduce under laboratory conditions at drinking water temperatures between 20 and 25°C. In field studies, *L. anisa* is also frequently found in drinking water sampled from the cold water portion of the plant, but *L. pneumophila* can also be found sporadically in cold water. However, it remains unclear to what extent these legionella bacteria have multiplied at drinking water temperatures below 25°C, because it is not clear what the drinking water temperature is in that part of the cold water system where multiplication occurs.
In any case, it is clear that the management measure contained in the current regulations that the cold water temperature
must be below 25°C is insufficient to allow propagation of *L. anisa* in particular in the cold water part of the tap water system. Thus, if the Regulation on Legionella Prevention in Drinking Water and Hot Tap Water continues to focus on all culturable *Legionella* spp, it is actually necessary to institute additional control measures when the drinking water has a temperature between 20 and 25°C, but currently effective control measures to achieve this are not available.

The advice based on the scientific evidence is to make the management plan for most priority institutions
focus on culturable *L. pneumophila* rather than culturable *Legionella* spp. Because monitoring is a part of the management plan, it is therefore also recommended that monitoring at these priority institutions be focused on *L. pneumophila*, for this purpose it is necessary to have a specific and validated and standardized detection method for *L. pneumophila*
use. For priority settings where there is a high density of people with severely weakened immune systems (such as hospitals, for example), it is recommended that the management plan, including monitoring, continue to focus on culturable *Legionella* spp, in accordance with current Dutch legislation (including the transposition of the new Drinking Water Directive).

A modification in line with the above will affect laboratories, building managers, installation managers, legionella prevention advisors (BRL 6010) and enforcers (ILT).

CHAPTER 8

Risk volume less than one liter

8.1 Current legislation

These rules apply only to priority institutions.

In the Regulation for the prevention of legionella in drinking water and warm tap water, the following is included in article 5: *'The owner of a collective water supply or collective piping system as referred to in section 35(1) of the Decree, shall carry out a legionella risk analysis in accordance with the requirements laid down for this in Annex 2 to these Regulations, and shall ensure that measures are taken in this respect.* In section 5.2 'Risk factors and risk qualification to be used in the risk analysis' of Annex 2, a table is included with risk qualifications of various risk factors related to the legionella risk analysis.
to the drinking water temperature. Under this table, the following is included as a note: *'For pipe volumes smaller than one liter, for all temperatures above 25°C the risk rating is neutral (0), provided there is a good flow'.* In addition, it is prescribed that when sampling, the first liter must be flushed away, and then the sample may be taken.

This is known as the so-called "1-liter rule. This rule is based on the assumption that the likelihood of contamination of humans through exposure to such small volumes is
is negligible. For this reason, a neutral risk is attributed to pipes with a volume less than one liter.

8.2 Reason to include topic in evaluation

Recent publications have questioned whether pipe volumes of less than one liter provide a neutral risk for *Legionella (*Van der Lugt *et al.* , 2019; Nuijten, 2019). Also
has been indicated in a previous report that this 1-liter rule is one of the reasons why lowering the hot tap water temperature in homes can be seen as a neutral risk for *Legionella* propagation (Van Wolferen, 2019). In addition, the thermostatic
mixer and the pipe and end (e.g., shower head) after the thermostatic mixer to the 1-liter rule, so thermal management need not be applied to this part of the tap water system. Showers with thermostatic mixing valves are used almost universally in healthcare facilities and hotels, among other places. Several members of the Guidance Committee also indicated that it was unclear to them whether a pipe volume less than one liter posed a neutral risk for Legionella. The one-liter rule appears to have been included in the legislation at the time as a practical measure be, but this measure does not seem to have been based on the scientific insights of the time. It is therefore important to examine whether this measure is in line with current scientific understanding for this topic as well.

8.3 Brief overview of scientific insights for 2001

The decision to provide a neutral risk rating for pipe volumes smaller than one liter, regardless of drinking water temperature, was made at the time on practical grounds. Thus, this neutral risk qualification for pipe volumes smaller than one liter was not driven by scientific understanding at the time.

8.4 Overview of scientific insights since 2001

To find out whether or not a volume less than one liter poses a risk for growth of *Legionella (pneumophila)*, the extent to which *Legionella* has been found in small tap water systems of houses, in the last meters of tap water systems (e.g., shower hoses, shower heads, thermostatic mixing valves) and in small pilot tap water systems with volumes less than one liter. These aspects are further explained and described below.

8.4.1 *Legionella* in tap water systems of homes

A report by Van Wolferen (2019) indicated that tap water systems of homes (i.e., no apartment complexes) generally have a maximum pipe length of 10 meters from the attic to the furthest kitchen tap point or washbasin (assuming a standard diameter of 10 mm) and a maximum pipe length of 6 meters from attic to furthest shower or bath faucet (assuming standard diameter 10 to 13 mm). A calculation of the content of such tap water installations shows that the content is always less than one liter. This included requirement in the regulations therefore implicitly means that the risk of *Legionella (pneumophila)* from residential tap water installations is almost always neutral (Van Wolferen, 2019). When studies have shown that growth of *Legionella* occurs in home tap water systems, it could be concluded, based on the aforementioned report (Van Wolferen, 2019), that *Legionella* has multiplied in a volume less than one liter. However, several members of the guidance group indicated that the assumptions made in Van Wolferen's report regarding pipe length and diameter for many homes were incorrect and that this length and diameter are normally larger, so that the volume of tap water systems in homes usually is greater than one liter (pers. comm. Eric van der Blom, Oscar Nuijten and Rick Langen). We have, despite the discussion whether the pipe volume in houses of private individuals is smaller or larger than one liter, identified studies where growth of *Legionella* in pipe water systems of houses has been investigated. It is therefore noted that this does not necessarily mean that the volume was less than one liter.

Many scientific studies show that home tap water systems may be colonized with culturable *Legionella* spp, including *L. pneumophila* (Wadowsky *et al.* , 1982, Arnow *et al.* , 1985, Lee *et al.* , 1988, Alary & Joly, 1991, Stout *et al.* , 1992, Mathys *et al.* , 2008, Den Boer *et al.* , 2015, Collins *et al.* , 2017, Dilger *et al.* , 2018, Hayes-Phillips *et al.* , 2019). In addition, studies have also been published that have shown an epidemiological link between the *L. pneumophila* strain isolated from the patient and from the tap water system of the patient's home (Stout *et al.* , 1987, Chen *et al.* , 2002, Luck *et al.* , 2008, Den Boer *et al.* , 2015). This last study is specifically about the Dutch situation. Some of these studies also attempted to identify risk factors that were significantly related to the number of legionellapositive tap water systems in homes of individuals. The results of these studies showed that the diameter of the piping of the tap water system or the volume of the boiler tank did not significantly affect *Legionella (pneumophila)* in the tap water systems of homes of individuals (Alary & Joly, 1991, Stout *et al.* , 1992). Thus, in those studies, there appears to be no relationship between the volume in a home's tap water system and the rate of growth of *Legionella (pneumophila)* in the tap water system. An Italian study did find a relationship between system piping length and the presence of culturable *Legionella* (Borella *et al.* , 2004). When the length of the piping from the heating element to the tap was more than 10 meters, there appeared to be an increased risk that the system was positive for culturable Legionella. This could mean that larger volumes in a tap water system result in an increased risk of growth of culturable Legionella.
However, in addition to distance, the concentration of free chlorine was also found to be a significant risk factor in this Italian study. It is therefore plausible that with increasing pipe lengths in the Italian tap water systems studied, the concentration of free chlorine decreases and that this lower concentration of chlorine the reason why *Legionella* was more often found at distal points in tap water systems with long pipes. Mathys *et al.* (Mathys *et al.* , 2008) compared the data from their study of the presence of *Legionella* in

tap water systems from single-family homes with results from other studies that sampled tap water systems from large buildings. They conclude from this comparison that smaller tap water systems (from single-family homes) are less likely to be positive for culturable *Legionella* than larger tap water systems, but that the maximum number of culturable *Legionella* at ^{106} cfu/l in a small tap water system can be as high as in a large tap water system.

The same conclusion was also reached by another study, where drinking water from tap water systems of different buildings (from small tap water systems in houses to larger systems in apartment buildings, hospitals or hotels) in Cologne were sampled and analyzed for culturable *Legionella (*Kruse *et al.* , 2016).

From these studies, it can be concluded that culturable *Legionella* can also multiply in tap water systems of homes and that the maximum numbers found in a tap water system are similar are with tap water systems of large buildings. Although it remains unclear to what extent the tap water systems studied have a volume smaller than one liter had, these results do show that the volume of a tap water system does not show a clear relationship with propagation of *Legionella* in a tap water system.

8.4.2 *Legionella* in last meters of a tap water system

Many studies have shown that culturable *Legionella (pneumophila)* is not evenly distributed throughout a tap water system, particularly the last meter of distal taps are zones of the tap water system where the highest Legionella numbers are found (Schoen & Ashbolt, 2011, Kistemann & Wasser, 2018, Totaro *et al.* , 2018, Hamilton *et al.* , 2019). In the study by Kistemann & Wasser (2018), the results of routine taken drinking water samples for legionella monitoring for 7 years, a total of 30,000 drinking water samples taken from 4,600 public buildings in Germany, analyzed. The results showed that samples taken at distal taps without flushing were more often positive (18.8%) than samples taken after flushing (4.7%). Culturable *Legionella* thus increased mainly in the distal part of the pipe. Also, the study shows that centrally collecting and analyzing the results of routine monitoring programs for *Legionella* can provide a wealth of data on possible risk factors for culturable Legionella. Other studies have shown that the culturable legionella numbers in distal pipes were often higher by a factor of 10 than those in the recirculation loop of the tap water system (Cristina *et al.* , 2014, Totaro *et al.* , 2018, Bedard *et al.* , 2019) or that the number of positive legionella samples is higher at distal taps compared to central taps (Kruse *et al.* , 2016).

Several studies have also examined whether culturable *Legionella* are present in the biofilm that has developed in the last part of the installation (for example, in shower hoses, shower heads, and thermostatic faucets). Sampling of water from the shower head and of a small proportion of biofilm in the shower hose in single-family homes in Britain showed that in 40% of cases where the water from the shower head was positive for culturable *Legionella* (including *L. pneumophila*), the biofilm in the shower hose was also positive (Collins *et al.* , 2017). This study also showed that the proportion of positive shower hoses is related to the age of the facility and that the percentage of positive shower hoses increased significantly with the number of years the facility was in use. Other researchers also found *Legionella* spp in the biofilm of shower hoses that came from homes of individuals around the world (Proctor *et al.* , 2018). A study where thermostatic mixing faucets were disassembled and the biofilm of different parts of the faucet was sampled showed that the biofilm in these faucets can contain high numbers of culturable *Legionella*, with the highest numbers observed on the rubber parts (up to 1.8×104 cfu per swab)(van Hoof *et al.* , 2014). Thus, also in that study, the numbers of culturable *Legionella* spp in the water from the thermostatic mixing faucet were caused by the growth of *Legionella* on components in the faucet. Other studies have also found culturable *Legionella* in faucets at taps of a tap water system (Sydnor *et al.* , 2015, Lee *et al.* , 2018, Mazzotta *et al.* , 2020). In the latter two studies, it was also observed that the number of legionellapositive drinking water samples in hospitals decreased significantly when thermostatic mixing valves were replaced with manual mixing valves. Finally, it was also observed that cases of legionellapneumonia were related to *L. pneumophila* that were found in the last liter of the tap water system (Hamilton *et al.* , 2018).

The Netherlands also has case histories of patients linked to the presence of *L. pneumophila* in the last liter of a plant and some examples have been shared by RIVM. In these examples, genotypic matches were found between the clinical isolate of a patient and the isolate from a shower hose or shower head of a tap water system. In addition, a number of times
L. pneumophila found in the swab of a shower hose or the filling hose of a jaccuzzi when sampling potential sources of patients.

From the studies described in this section, it can be concluded that *Legionella (pneumophila)* can multiply in the distal part of a tap water system, where the volume to the endpoint is less than one liter.
In addition to the fact that the distal part of a tap water system can thus be positive for culturable *Legionella,* it is generally found that Legionella numbers in the distal part of the system are higher than in the central part of the system. This observation shows that the volume in the distal part of a plant is thus more likely to have an increased risk than a neutral risk. Finally, actual cases of illness that could be linked to legionella strains in the distal part of the tap water system are also known.

8.4.3 *Legionella* in small pilot tap water systems

In addition to these field studies, many studies have been published where growth of culturable *Legionella (pneumophila)* has been observed in pilot tap water systems (Van der Kooij *et al.* , 2005, Liu *et al.* , 2006, Farhat *et al.* , 2010, Rhoads *et al.* , 2015, Buse *et al.* , 2017, Rhoads *et al.* , 2017, Van der Kooij *et al.* , 2017, Van der Lugt *et al.* , 2017, Learbuch *et al.* , 2019, Bleys & Dinne, 2020). In almost all cases, these pilot tap water systems are relatively small and often have a volume of less than one liter. In addition, for a single study, *Legionella* has also been shown to multiply in the tap of the pilot tap water system (Van der Lugt *et al.* , 2017).

8.4.4 Dutch situation

The study on the occurrence of culturable *Legionella* in priority settings is the most comprehensive field study conducted in the Netherlands (Van der Lugt *et al.* , 2019). The authors conclude that the risk classification of 0 for tap water installations of small buildings, as included in the Dutch legislation, is not supported by the data found. Water from the tap water systems of small buildings had more than two times higher rates of exceeding the legal standard for *Legionella* in drinking water (100 cfu/l) than
water from tap water installations of large buildings. A caveat to this conclusion, however, is that only priority installations were included, so it is expected that the tap water installations of small buildings had a capacity of more than one liter and thus were not, as the
authors noted, had a risk rating of 0. Another study showed that high numbers of culturable *Legionella* were found on the rubber parts of thermostatic faucets from tap water systems of a hotel and hospital in the Netherlands (Van Hoof *et al.* , 2014). Thus, the positive drinking water samples from these faucets were probably not linked to the length of the tap water system, but to the use of materials in the thermostatic mixing valve. Legionella case studies further showed that disease cases in the Netherlands could be linked to the presence of *L. pneumophila* in, for example, shower hoses (pers. comm. Petra Brandsema RIVM). Finally, it has been found that *L. pneumophila*
can propagate to high numbers in a small pilot tap water plant (generally with a volume of less than one liter) that was fed with different drinking water types of the Netherlands (Van der Kooij *et al.* , 2017) or in the taps of a pilot tap water plant that was
fed with Dutch drinking water (Van der Lugt *et al.* , 2017).

8.5 Conclusion scientific state of affairs 1-liter rule

Review of the current state of scientific understanding of Legionella risk has shown that culturable *Legionella* spp may be present in the last part of
the tap water system, where the culturable Legionella numbers are often higher than in the central parts of the tap water system. In addition, propagation of *Legionella (pneumophila)* has been demonstrated in shower hoses, shower heads and faucets (especially thermostatic faucets) and succeeds well in small pilot installations with a volume of less than one liter. Finally, disease cases are known in the Netherlands where the patient's Legionella strain gave a match to the Legionella strain isolated from the shower hose. From the current state of knowledge on *Legionella*, it is therefore concluded that volumes smaller than one liter are also a risk for spreading *Legionella (pneumophila)*.

8.6 Knowledge and experiences from practice

The '1-liter rule' was a practical approach from the Health Care Inspectorate and KWR during a consultation (at the time) at the Ministry of VROM, which was related to the '3-liter rule' used in Germany. It was then decided to be stricter in the Netherlands. The '1-liter rule' was drawn up with a view to the (cold water) connecting pipe of a hot water supply. Due to heat conduction from the hot water preparer, there is always locally favorable growth conditions in the cold water pipe. This is an unavoidable situation. In fact, without it, you would not be able to use hot water systems. It was stated at the time that the combination of a small volume and a good guaranteed flow will lead to, at most, low legionella concentrations at that position and thus a relatively low risk. Practice has more or less shown that this has also hardly led to any problems. The '1-liter rule', which is in fact arbitrarily chosen, is frequently and sometimes 'conveniently' used in daily practice. Think of the installation of pipes with a smaller diameter over a long length to a tap point to stay within the one liter. It is questionable whether that rule was intended for that purpose, especially since we are not sure how risky these types of situations are. Also
in the ISSO Publication 55.1 and other guidelines, that rule has received a lot of elaboration. Deleting such a rule is going to have a lot of impact in the daily practice of legionella prevention and management. If scientific insights make it necessary to modify the rule, you could make it more specific.

A number of respondents indicated that the "1-liter rule" is wrongly interpreted so broadly. The legionella risk may be precisely behind the thermostatic mixer, also due to the materials used there, think of the shower hose. But also in thermostatic shower mixers themselves a lot of biofilm is found as shown by practical research in a preliminary study of TVVL/ISSO ST-32.
The risks of mixing valves, shower hoses and shower heads must be considered. This should not be dismissed as a neutral risk. We do not know enough about what happens in the first liter because it is washed away when samples are taken. The practical experience of one respondent does show that most instances of non-compliance with standards (75%) can be related to the last part of the outlet system behind the shower mixer. In addition to shower mixers, dishwashing or pre-rinsing showers also give rise to many legionella problems.

8.7 Recommendation to adjust regulations based on scientific insights

The current Regulation on Legionella prevention in drinking water and hot tap water states that for pipe volumes smaller than one liter for all temperatures above 25°C the risk qualification is neutral (0) (see section 8.1).
This rule is at odds with current scientific knowledge and practical experience, because several studies and practical situations have shown that the last part of the tap water system (for example, from thermostatic mixing valve to shower head) also poses a risk of spreading *Legionella* and there have even been cases of disease
observed that could be traced to the last part of a tap water system. In daily practice, the experience is also that *Legionella* is found with some regularity in the last part of the installation, where the
volume is less than one liter. Interviews revealed that this one-liter rule was included in the legislation at the time because the pipe from cold water supply to the hot water heater heats up under the influence of the hot water in the hot water heater, but that such pipes are not
can be treated with thermal management. This means that if such a regulation were not included in the regulations, it would no longer be possible to construct hot water systems.

The study in Great Britain has shown that during the first few years of shower hose use, the number of positive shower hoses increases significantly. Based on that observation, it might also make sense to recommend for priority settings that shower hoses and shower heads be replaced every three to five years. However, it is necessary to first find out whether in the Dutch situation age of the shower hose is also a risk factor to test positive for culturable *Legionella*. Then the optimal duration before replacing shower hoses, shower heads and possibly (thermostatic) mixers can be determined.

The advice based on the scientific findings is to keep the exemption of components in
tap water systems with pipe volumes of less than one liter, as described in section 5.2, appendix 2 of the Regulations on the prevention of Legionella in drinking water and domestic hot water, and to include a separate rule that the connecting pipe of the domestic hot water system (from the cold water system) is not regarded as a risk factor, provided there is sufficient flow. Also, when sampling, the first liter should be sampled and no longer flushed away as is currently prescribed.

An adaptation in line with the above has a rather large consequence for performing risk assessments the practice. For example, the risk assessment of chilled water and hot water outlet pipes in the ISSO Publication
55.1 partly based on the content of the outlet pipe (≤ 1 liter or > 1 liter). In the current situation, this means that at a 24-hour average indoor temperature of > 25 °C, the situation with a pipe volume of ≤ 1 liter is assessed as risk-neutral provided that there is at least weekly use. Letting the '1-liter-rule' lapse means that the distinction according to pipe volume can no longer be made and that the risk assessment of outlet pipes prescribed in ISSO 55.1 must change. In essence this means a simplification of the risk assessment methodology, but it does lead to more outlets with a negative risk rating. Both science and practice indicate that the latter is justified. However, it should be noted that this removes the incentive to limit the length of drain pipes as much as possible. NEN 1006 contains also no requirements regarding the length of drain pipes. A modification in line with the above will affect legionella prevention advisors (BRL 6010), building managers, installation managers, NEN standards subcommittee NEN 1006.

CHAPTER 9

Risk qualification of collective water supply or piping network

9.1 Current legislation

These regulations apply only to priority institutions.

Section 5.2 of Annex 2 of the Regulation on Legionella Prevention in Drinking Water and Hot Tap Water contains a risk qualification table for the risk management plan to be drawn up for priority establishments.

9.2 Reason to include topic in evaluation

In the current risk rating, only temperature (in combination with flushing) and pipe volumes greater than one liter are included as risk factors, while the above sections show that other factors (e.g., pipe material use, pipe volume less than one liter) can also be a risk factor. In addition, the members of the supervisory committee indicated that other risk factors (e.g. faucet type) may also play a role in the propagation of *Legionella* in tap water systems. Based on these observations, it is important to also review the risk classification table in relation to current scientific knowledge about Legionella.

9.3 Brief overview of scientific insights for 2001

The risk classification of the collective piping networks of priority buildings is based on the water temperature and whether the installation has a pipe volume smaller than one liter. The scientific origin of the temperature above 45 to 50°C is described in section 3.2. The scientific origin of the risk ratings for temperatures <25°C and 45°C is based on several publications, which have shown that *Legionella* pneumophila is able to multiply at drinking water temperatures between 25 and 45°C (Tison *et al.* , 1980, Yee & Wadowsky, 1982, Wadowsky *et al.* , 1985, Kusnetsov *et al.* , 1996). The original rationale for applying a heat shock procedure is also described in Section 3.2. As described in Section 8.2, there is no scientific justification for the risk rating of neutral for pipe volumes smaller than one liter.

9.4 Overview of scientific insights since 2001

9.4.1 Influence of temperature on die-off of *Legionella* (pneumophila)

Sections 3.3.1 and 3.3.2 describe the latest scientific findings regarding the effects of drinking water temperatures higher than 45 to 50°C on *Legionella (pneumophila)* die-off and the effect of heat shock treatment on *Legionella* (pneumophila) die-off.

9.4.2 Influence of temperature on growth of *Legionella (pneumophila)*

Since 2001, few new insights have appeared regarding the influence of temperature on growth of *Legionella* (pneumophila). In particular, studies have been published that have confirmed that *L. pneumophila* is able to reproduce between 25 and 45°C for example (Ohno *et al.* , 2008, Buse & Ashbolt, 2011, Van der Kooij *et al.* , 2016, Buse *et al.* , 2017). A study investigating the influence of temperature on growth of *L. anisa* and *L. pneumophila* in biofilms of a pilot tap water system showed that *L. pneumophila* barely multiplied at 25 to 33°C, while *L. anisa* multiplied to relatively high numbers in the biofilm (Figure 8) (Van der Kooij *et al.* , 2009).

At a temperature of 34°C, *L. pneumophila* and *L. anisa* were both found to multiply to high numbers. Thus, these results show that growth of *L. pneumophila* occurs at 25 to 40°C, but above 33°C *L. pneumophila* was able to multiply to higher numbers than below 33°C. Scientific research has also shown show that the temperature range at which *L. pneumophila* can reproduce depends on the strain or sequence type of *L. pneumophila* (Buse & Ashbolt, 2011; Van der Kooij *et al.* , 2016). Practical studies have shown that in Israel cultivable *L. pneumophila* in tap water systems dominated the culturable legionella population and was found in relatively high numbers (~1×103 cfu/l) at temperatures between 27.5 and 29.1°C (Rodriguez-Martinez *et al.* , 2015) and that on three Caribbean islands culturable *L. pneumophila* also dominated the culturable legionella population in distributed drinking water with relatively high numbers (~1 ×104 cfu/l) at a temperature range of 28 to 30°C (Valster *et al.* , 2011). This is consistent with the general notion that *L. pneumophila* is particularly able to reproduce between 25 and 45°C (National Academies of Sciences, 2019).

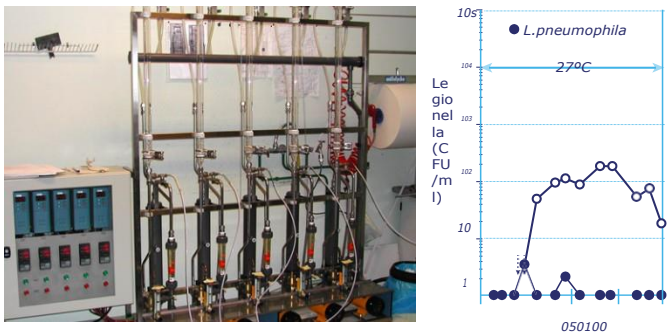


Figure 8. **The numbers of *L. pneumophila* and *L. anisa* in cfu/ml as a function of the number of days (right) developed in a drinking water pilot plant (left). Source: Van der Kooij *et al.* , 2009.**

9.4.3 Risk factors other than temperature

The scientific literature has also shown that in addition to temperature, other factors also provide an increased risk for growth of *Legionella (pneumophila)*. For example, Chapter 6 concluded that application of materials such as PVC-P, rubber and PE materials in a tap water system promotes the growth of *Legionella* and can lead to higher numbers of culturable *Legionella* in the tap water system. Also, a committee of international Legionella experts recently concluded that electric water heaters, thermostatic mixing valves, and electronic faucets can increase the risk of *Legionella* growth in tap water systems (National Academies of Sciences, 2019). For example, studies have shown that increased numbers of culturable *Legionella* spp are present in the biofilm developed on surfaces of thermostatic mixing valves (Van Hoof *et al.* , 2014, Van der Lugt *et al.* , 2017).

The study by Van der Lugt *et al.* (2017) also showed that thermostatic brass faucets were more likely to be positive for *L. anisa* and had higher numbers than a ceramic faucet or a stainless steel faucet. Studies have also shown that electronically activated faucets have a higher risk of being positive for culturable *Legionella (*Halabi *et al.* , 2001, Sydnor *et al.* , 2015). The results showed that water from 95% of electronic faucets was positive for culturable *Legionella* at least once, while water from 45% of manual faucets was positive, which was a statistically significant difference ($p<0.01$) (Sydnor *et al.* , 2015). Electronic faucets contain thermostatic mixing valves and complex aerators that lower the flow rate. The increased bacterial growth observed in electronic faucets was caused by the lukewarm water temperature and reduced flow rates and it is likely that these factors also promote legionella growth (Charron *et al.* , 2015, National Academies of Sciences, 2019).

A recent study examined whether the orientation of the pipes running from a recirculating hot water pipe to a tap point affects the risk for growth of *L. pneumophila (*Rhoads *et al.* , 2016). The hypothesis here is that in ducts that are oriented downward (in other words, running from the recirculation loop in the ceiling to the tap) no convection mixing occurs, while in pipes that run horizontally or are oriented upward does occur convection mixing, leading to warming of the stagnant water in the distal pipe. This warming could then lead to increased growth of *L. pneumophila*.

The researchers constructed a pilot tap water system in which pipes were oriented downward and upward from a recirculation loop containing hot water. The results showed that the temperature in the distal pipes oriented downward cooled to room temperature (23-24°C) within 30 minutes and after 30 cm of the recirculation loop, regardless of the hot water temperature. However, the distal pipes oriented upward had a temperature of 30.2°C after 30 minutes when the hot water temperature of the loop was 40°C and 38.8°C when the hot water temperature of the loop was 58°C.
During stagnation, the temperature in this upwardly oriented distal pipe decreased to 42°C after 30 cm and decreased to 30°C until the end of the pipe (total pipe length was 1.5 meters). These temperature profiles show that unlike downward oriented pipes,
convection mixing occurs in upward-facing pipes and that this leads to temperature ranges that are ideal for growth of *L. pneumophila* during stagnation.

Therefore, the numbers of *L. pneumophila* in the drinking water were significantly ($p<0.05$) higher in the distal pipes oriented upward than those oriented downward. In the plant that was minimally flushed (1 flush per week), the numbers of *L. pneumophila* in the drinking water increased 3.5 logen units in the pipes oriented upward relative to the numbers in the recirculation loop, whereas for the pipes oriented downward this was
being oriented downward was 2.8 logen units. When the plant was flushed more frequently (three flushes per week), this difference between upward and downward oriented pipes was no longer observed. Interestingly, the numbers of *L. pneumophila* in the biofilm were below the detection limit in all cases, regardless of the orientation of the distal pipes. This last observation makes it difficult to explain where *L. pneumophila* was able to reproduce in the system, also because *L. pneumophila* was determined
with qPCR (which does not distinguish between dead and live Legionella cells), leaving it unclear to what extent the *L. pneumophila* found in the drinking water from the distal pipes are also viable. In addition, no other scientific studies were found that examined the influence of convection mixing in pipes of a tap water system on growth of *Legionella (pneumophila)*.

9.5 Conclusion scientific state of affairs influence of temperature

From the above scientific insights, it is concluded that in addition to temperature, the use of piping materials and type of faucets are also risk factors
are for propagation of *Legionella (pneumophila)* in tap water systems. Conclusions concerning hot water temperature as a risk factor are described in section 3.5, those concerning piping materials in section 6.5. Scientific studies over the past 20 years on the temperature range at which *L. pneumophila* is able to reproduce have confirmed that *L. pneumophila* can reproduce particularly between 25 and 42°C.
L. anisa is already able to multiply to high numbers at temperatures of 25°C or lower. Increased risk of growth of *Legionella (pneumophila)* also applies when thermostatic mixing valves or electronic faucets are used instead of manual faucets with no mixing chamber in the faucet. Finally, one study showed evidence that the flow direction of distal pipes also increases the risk of growth of
L. pneumophila can increase, with upward-oriented pipes having an increased risk (due to convection mixing) of Legionella growth than downward-oriented pipes (where no convection mixing occurs). However, it remains unclear whether this finding also applies to culturable *Legionella,* Dutch drinking water and in field situations and in what way *L. pneumophila* manages to propagate in upwardly oriented pipes, as they were not detected in the biofilm.

9.6 Knowledge and experiences from practice

One of the respondents indicated that the table with temperature and tool life that we use may need to be adjusted based on the latest scientific insights. This would then amount to higher temperatures and longer standing times. This is partly based on the conclusions from the study by Van Kenhove (2018). (This study was discussed in Chapter 3).
The disadvantage of the current risk rating table with pluses and minuses is that in practice it is interpreted very black and white with too much focus on temperature. There are more relevant risk factors besides temperature. In addition, the assessment at higher temperatures is not correct and needs to be modified.
The influence of material choice on legionella growth should receive more attention. In the existing ISSO 55.1, for example, materials are not reflected as distinctive in the risk analysis.

9.7 Recommendation to adjust regulations based on scientific insights

Sections 3.5 and 6.5 of this report have already indicated in more general terms what the current scientific state of affairs means for the regulation of
of thermal management and piping materials in the legislation. In addition, the use of thermostatic mixers and electronic mixers appears to have an increased risk for propagation of culturable *Legionella*. Some of these risk factors are not currently accounted for
or correctly taken into account in the risk qualification of tap water installations as included in the legislation (Table in 5.2, of Annex 2 of the Regulation on the prevention of Legionella in drinking water and warm tap water). In that particular table However, it did include that heat shock treatment would be an effective measure to kill off culturable *Legionella* spp and that a pipe volume smaller than one liter would be a neutral risk, even if the temperature is in the optimal range for growth of culturable *Legionella (pneumophila)*. However, the description of the scientific literature in Chapters 3 and 8 of this report has shown that these passages in the risk classification table are not in line with current scientific knowledge.

The provisional recommendation, based on scientific insights, is to replace the current table in paragraph 2, section 5 of the Regulation on 'Legionella prevention in drinking water and warm tap water' with a table in which several known risk factors are briefly included. This should in any case include temperature, pipe material and tap type.

A modification in line with the above will affect legionella prevention consultants (BRL 6010), building managers, installation managers.

B

CHAPTER 10

Answering questions of evaluation framework and conclusions

In this chapter we answer - based on the previous chapters - the central question, the subquestions and the questions from the evaluation framework. We begin, in line with the evaluation framework, with a summary of the line of reasoning using the IAK questions. We begin with a summary problem analysis and objective by answering the IAK questions 14. In doing so, we focus from IAK question 3 on the problems that may require policy adjustment. Then we describe the way the government can intervene to solve the problems and achieve the objectives by answering the IAK

questions 57. We then use this line of reasoning to answer the stated central question and sub-questions one by one in the third section of this chapter.

10.1 Summary problem analysis and objective (IAK questions 1-4)

10.1.1 IAK question 1: what is the trigger?

After the 1999 Legionella outbreak in Bovenkarspel, requirements were set to prevent new Legionella outbreaks based on the state of the art at the time. An important part of these requirements focused on the prevention of *Legionella* in drinking water and hot tap water systems. Thereby, after the temporary regulation focused on all collective tap water systems, the preventive efforts were
on priority establishments. Based on the Drinking Water Decree, these institutions were obliged to meet additional requirements in addition to the current obligations in the Building Decree 2012 (and underlying regulations) and NEN 1006. The legislator opted for these additional requirements because it was expected that in the priority settings many people from the infection risk group could come together and/or that the government has a duty of care in these settings.
These additional requirements, in short, require priority establishments to prepare a legionella risk analysis and - if the risk analysis warrants it - to prepare and implement a legionella management plan. These legionella prevention measures should focus on aerosol-forming taps.

Since 1999 a lot of research has been done on Legionella. There is more knowledge about the conditions under which *Legionella (pneumophila)* grows (or does not grow), which Legionella species are dangerous to humans and how *Legionella* can best be controlled and prevented. In order to achieve regulation
to come up with requirements that actually lead to the prevention of *Legionella* infections, it is important to regularly check whether there are any new scientific insights that require adjustment of the regulations. This report answers this question. It is also important for answering the IAK questions that a new Drinking Water Directive was published at the beginning of 2021, which contains regulations on Legionella prevention and which must be transposed into Dutch law by the beginning of 2023 at the latest.

10.1.2 IAK question 2: What is the force field?

Dutch policy aims to prevent people from becoming ill or dying as a result of *Legionella* infections. This policy goal is undisputed, both in parliament and in society at large.
To achieve this policy goal, a system has been set up in which different parties play a role and regulations have been made to ensure that the parties contribute properly to the policy goal.

1. *System Responsibility*

The Minister of IenW (or the State Secretary, depending on the portfolio allocation) - the core department - is system responsible for drinking water. This means that the ministry monitors the effects of the policy, investigates and evaluates. If it turns out that there is a problem, this can lead to a proposal to amend the law or to adjust the conditions (finances or expertise promotion) under which the implementation of the policy takes place. In addition, the Ministry of Health has a responsibility for detecting and dealing with infectious diseases, such as legionellosis.

2. *Implementation*

The implementation of the policy lies with various system parties. First responsible for implementing the policy is the
person who owns drinking water and
hot water systems. These owners must ensure that these systems are compliant. Additional requirements apply to systems in priority settings.
In addition to the owners of these systems, other parties play a role. These include:

- Anyone involved in the installation and maintenance of a system (from architect to technician)
- BRL-6010 certified companies
- the GGD, the Streeklab Haarlem and other laboratories
- Parties involved in the formulation of NEN standards, including NEN 1006.

3. Supervisor

The supervision of compliance with the rules for the prevention of *Legionella* in drinking water and hot tap water systems is vested in the Human Environment and Transport Inspectorate (ILT) (for priority institutions) and the Municipal Executive (for all buildings pursuant to the 2012 Building Decree). In carrying out the inspections
– for priority and non-priority establishments - the water companies play an important role on the basis of Article 24 of the Drinking Water Act. This inspection task is carried out on a risk basis and relates not only to the (public-law) regulations but also to the (private-law) connection conditions of the water companies themselves. If the water companies find that the regulations have been violated, they inform the competent authority.

4. Stakeholders

The main stakeholders are the people who use drinking water and hot tap water systems. Sometimes these people also own the drinking water and hot water system, but in many situations this is not the case, such as in prisons, schools, nursing homes and hospitals. Certainly vulnerable people - who may be at a
legionnaires' disease more quickly become seriously ill - have a strong interest in ensuring that systems meet all requirements, even when they do not own a system. The Veterans Disease Foundation, among others, is an important representative of these stakeholders.

10.1.3 IAK question 3: what is the problem?

Current *Legionella* prevention policies are based on scientific assumptions from the beginning of this century.
Since then, scientific research has continued and the legionella policy deviates - in parts - from the scientific insights. Chapters 3-9 list some of these new scientific insights. In the process, requirements were found that are no longer in line with the state of the science. These requirements can be divided into four categories:

1. *Ineffective requirements:* these are requirements that have been determined to make no significant contribution to preventing human illness or death from Legionella infections:
   a. (see Chapter 7) our conclusion based on scientific evidence is that it is more effective to have the process of risk analysis, management plan and monitoring to focus on *L pneumophila*. This is the most dangerous species within the genus of *Legionella*.
2. *Requirements with a counterproductive effect:* these are requirements that can lead to more people becoming ill or dying from Legionella infections:
   a. (see chapter 8) an exception currently applies to drinking water and heat water systems with pipe volumes of less than one liter. In this situation the risk classification neutral (0) is used for all temperatures above 25°C, provided there is a good flow. This means that no control measures need to be taken for these pipes. Based on current scientific knowledge, there are no indications that the risk of *L. pneumophila* is smaller for pipe volumes of less than one liter. The absence of management measures for these pipeline volumes creates a risk of contracting a *Legionella* infection. The exception is therefore counterproductive.
   b. (see Chapter 3) a weekly heat-shock treatment with the given temperatures and standing times is according to current scientific knowledge and practical experience, not a reliable preventive measure to control Legionella (pneumophila) if
   the hot water temperature is below 60°C in the return pipe, at the mixing unit or at the tap point the measure may even result in increased Legionella numbers after the heat shock treatment due to growth of Legionella (pneumophila) on the dead biomass (released after heat shock treatment).
3. *Unproven requirements:* these are requirements that cannot be scientifically proven to contribute to the prevention of Legionella:
   a. (see Chapter 5) based on the current scientific literature, no statement can be made as to the extent to which weekly flushing of unused taps is a successful preventive strategy to control culturable *Legionella* in Dutch drinking water (distributed without disinfection residue) from tap water systems (on curative effects: see requirements with adverse effect).
4. *Missing requirements:*
   a. (see Chapter 6) the current scientific state of the art on the influence of piping materials on growth of *L. pneumophila* in drinking water systems shows that piping materials can have a significant influence.
   b. (see Chapter 9) when making the risk analysis, some of the risk factors described above are not taken into account or are taken into account correctly in the risk qualification of tap water systems.

10.1.4 IAK question 4: what is the goal?

We described under IAK question 2 that preventing people from becoming ill or dying as a result of infections with *Legionella* is an undisputed goal. In other words, there is support in politics and in society at large for legionella prevention provided there is sufficient benefit and necessity for the end user. The various parties to the system (see ICAO Question 2) have a role to play in realizing legionella prevention. Based on this division of roles, all system parties put people and resources into realizing the policy goal. For example, priority institutions employ people to carry out management measures, there are certified legionella advisers in accordance with BRL 6010, there are installers active who construct installations in line with the regulations and the Veterans' Disease Foundation brings people on the ground advocating for the interests of patients and at-risk groups.

To achieve Legionella prevention, it is crucial that all system parties not only fulfill their respective roles, but also do the right things. In other words, that the people and resources that system parties deploy to prevent *Legionella* are effective (efficient). For example,
That consultants performing a risk analysis also have a good focus on the biggest legionella risks in a tap water system.

A prerequisite for effective deployment of people and resources for the prevention of *Legionella* is that good requirements are set for the efforts of implementing system parties. Based on the above problem analysis (see IAK question 3), the conclusion is justified that some requirements are not good. These are the non-effective requirements mentioned above, the counterproductive requirements and the unproven requirements.
Furthermore, there are requirements that are missing. These requirements (or the lack of them) mean that the people and resources deployed by implementing system parties are not always used effectively.

The goal based on the scientific analysis and the resulting problem analysis (IAK question 3) is therefore:

To adapt - in line with the latest scientific insights - the requirements for the implementation of legionella prevention in order to strengthen the effectiveness of the implementation of legionella prevention in drinking water and hot tap water systems.

10.2 Summary analysis of government intervention and the consequences thereof (IAK questions 5-7).

The - in line with the latest scientific insights – Adjusting the requirements for implementing legionella prevention in order to increase the effectiveness of implementing legionella prevention in drinking water and strengthen hot water systems is significant for all system parties. In this summary analysis, we make this significance more concrete by answering IAK questions 5-7.

10.2.1 IAK question 5: what justifies government intervention?

Whether modifying implementation requirements warrants government intervention depends on how these requirements were set. For example, when requirements follow from NEN 1006, government intervention may not be the most appropriate route. We therefore begin the answer to IAK question 5 with an overview of where the requirements from the problem analysis (IAK question 3) are found in the regulations and what is needed to bring them in line with the latest scientific insights.

An overview of requirements that are no longer in line with the state of the art

In the table below, we summarize the ineffective, counterproductive, unproven, and missing requirements:

Issue	Scientific adjustment?	assessmentWhere	Who is	
Target only L. pneumophila in most priority settings (see detailed Chapter 7 and IAQ Question 3 under 1a)	Not purposeful	Article 4, paragraph one, of the Regulation on Legionella Re vention	The Minister of IenW	The proposal is to differentiate within priority institutions (see further section 7.7 and section 10.2.2).
The 1liter rule (see expanded main piece 8 and IAK question 3 under 2a)	Adverse Effect	Appendix 2 Regulation of legionella laprevention	The Minister of IenW	The 1liter rule is an exception.
Application of heat shock as a prevention ve management measure (see detailed chapter 3 and IAK question 3 under 2b)	Adverse Effect	• Appendix 2 Regulation legio nella prevention • NEN 1006	• The Minister of IenW • NEN standards sub committee	• Scheme applies only to priority institutions. • Both for residential installations and other buildings. Coils must be distinguished
Preventive flushing of drinking water ter and hot water systems (see in detail chapter 5 and IAK question 3 under 3a)	UnprovenAnnex	2 of the Regulation legionella prevention	The Minister of IenW	of thermal disinfection. Flushing as a curative measure is counterproductive.
The influence of piping materials (see in detail Chapter 6 and IAK Question 3 under 4a)	Missing requirement s	Regulation materials and chemicals drinking water and hot water supply Appendix 2 Regulation on Legionella prevention	The Minister of IenW	Covers both material use requirements and requirements for risk analysis.
The table in 5.2, of Annex 2 of the Legionella Prevention Regulations lacks important risk factors.	Missing requirement s	Appendix 2 Regulation of legionella laprevention	The Minister of IenW	The current risk table also includes management measures. Important to take this into account when making adjustments.

Justification for government intervention? The requirements that are no longer in line with the state of the art are mainly in the Legionella Prevention Regulations. The Minister of IenW (or the State Secretary, depending on the portfolio allocation) has the authority to adjust this regulation (of course within the applicable legal bases). This means that the purpose - to adjust the requirements for the implementation of legionella prevention in order to strengthen the effectiveness of the implementation of legionella prevention - can only be possible through ministerial intervention and thus government intervention is justified.

The two adaptations to NEN1006 require a different process in which government organizations can play an encouraging role or determine to no longer refer to NEN standards in regulations. The adaptation of NEN standards does not fall within the scope of the study and we will therefore leave it out of consideration in the rest of the analysis.

10.2.2 IAK question 6: What is the best instrument?

The question of what is the best instrument to achieve the objective mentioned under IAK question 4 requires a balancing act. Four themes play a role in this consideration:

- Legality (what room does the national and international legal frameworks allow?).
- Effectiveness (by what means is the goal effectively achieved?).
- Efficiency (by what means is the goal achieved in an efficient way?).
- Feasibility.

In this section, we analyze the proposal according to these four themes. Then we answer the question of what is the best instrument.

Lawfulness

	IssueAttaches tolegality
Target only L. pneumophila in most priority settings (see detailed Chapter 7 and IAQ Question 3 under 1a)	In December 2020, the new Drinking Water Directive (2020/2184/EU) was published. In this directive, the distinction between pneumophila and nonpneumophila is not made explicit. It is therefore questionable whether the distinction proposed in this report is in line with the new directive. To obtain certainty about this, contact with the Committee (for example, via the process described in article 24, paragraph 2, of the guideline) is the most appropriate course of action. It is our assessment that making this distinction falls within the policy discretion of the member states.
The 1liter rule (see expanded chapter 8 and IAK question 3 under 2a)	No specific areas of concern.
Application of heat shock as a preventive management measure if hot water temperature is below 60°C (see detailed chapter 3 and IAK question 3 under 2b)	No specific areas of concern.
Preventive flushing of drinking water and hot water systems (see expanded main piece 5 and IAK question 3 under 3a)	No specific concerns
The influence of piping materials (see in detail Chapter 6 and IAK Question 3 under 4a)	There are pipe materials that are produced in other EU member states. It is important that new requirements for piping materials are compatible with rules that apply within the EU or between member states.
The table in 5.2, of Annex 2 of the Legionella Prevention Regulations lacks important risk factors.	No specific points of interest.

Effectiveness

	IssuePoints ofEffectiveness
Target only L. pneumophila in most priority settings (see detailed Chapter 7 and IAQ Question 3 under 1a)	This measure is proposed to focus monitoring on L. pneumophila because this species is the most dangerous to humans. At the same time, removing it outright is not effective in certain circumstances. In priority settings where there is a high density of people with severely weakened immune systems (e.g., nursing homes), monitoring culturable Legionella spp has a function because these people are also susceptible to less virulent Legionella species. It is not effective to consider Legionella nonpneumophila as an indicator of the presence of L. pneumophila.
The 1liter rule (see expanded chapter 8 and IAK question 3 under 2a)	This rule is counterproductive: thus the effectiveness of its removal is a given.
Application of heat shock as a preventive management measure if hot water temperature is below 60°C (see detailed chapter 3 and IAK question 3 under 2b)	The application of thermal disinfection, if the hot water temperature is below 60°C, is not effective as a preventive measure and may be counterproductive. It is more effective to prescribe other control measures for the prevention of Legionella.
Preventive flushing of drinking water and hot water systems (see expanded main piece 5 and IAK question 3 under 3a)	It is uncertain - based on the latest scientific insights - whether flushing is effective as a control measure for preventing Legionella. There is an efficiency issue here (see below).
The influence of piping materials (see in detail Chapter 6 and IAK Question 3 under 4a)	The current scientific state of the art on the influence of piping materials on growth of L. pneumophila in drinking water systems shows that piping materials can have a significant influence. This is also observed in practice. It is therefore effective to prescribe piping materials that are not above 400 pg ATP/cm2.
The table in 5.2, of Annex 2 of the Legionella Prevention Regulations lacks important risk factors.	Completing the missing requirements leads to better risk assessments and encourages the use of appropriate management measures. This increases effectiveness.

Efficiency

	IssuePoints ofeffectiveness
Target only L. pneumophila in most priority settings (see detailed Chapter 7 and IAQ Question 3 under 1a)	Much depends on the effectiveness of the methods available to only L. pneumophila to be measured (see further feasibility).
The 1liter rule (see expanded chapter 8 and IAK question 3 under 2a)	Based on the scientific evidence, the expectation is justified that dropping the 1-liter exception will lead to better risk analysis and therefore make management measures more effective than they are now.
Application of heat shock as a preventive management measure if hot water temperature is below 60°C (see detailed chapter 3 and IAK Question 3 under 2b)	No specific concerns. No longer using thermal disinfection as a preven tive measure provides room to apply other, better preventive management measures.
Preventive flushing of drinking water and hot water systems (see expanded main piece 5 and IAK question 3 under 3a)	From an efficiency point of view, it would be preferable to at least investigate whether the efforts of implementing parties to meet the flushing obligation could be better directed towards other management techniques. A prerequisite is that more effective preventive management measures are substituted.
The influence of piping materials (see in detail Chapter 6 and IAK Question 3 under 4a)	The distinction between new buildings/renovation and existing buildings plays a role in efficiency and feasibility. In new/renovated buildings it is easier to prescribe and therefore apply new materials than to replace pipe materials in existing buildings.
The table in 5.2, of Annex 2 of the Legionella Prevention Regulations lacks important risk factors.	It is true that better risk analysis leads to more appropriate management measures, which are more effective.

Feasibility

	IssueAttaches tofeasibility
Target only L. pneumophila in most priority settings (see detailed Chapter 7 and IAQ Question 3 under 1a)	There was (because it did not fit into the scope of the assignment) a limited examination of whether specific detection methods for L. pneumophila are described in the scientific literature. In any case, specific detection methods for L. pneumophila seem to be available and in some countries these methods have also been described as a standardized method. An additional litera ture and field study on possible specific detection methods for L. pneumophila are needed before a recommendation can be made on effectiveness and feasibility of such a method.
The 1liter rule (see expanded chapter 8 and IAK question 3 under 2a)	This measure leads to the adjustment of the risk analyses and subsequently to an adjustment of the management measures. It is expected that this will require an additional investment in the beginning, but in the longer term it will lead to more effective and efficient prevention of Legionella.
Application of heat shock as a preventive management measure if hot water temperature is below 60°C (see detailed chapter 3 and IAK question 3 under 2b)	In general, when this management measure expires as a preventive measure, other preventive management measures must replace it.
Preventive flushing of drinking water and hot water systems (see expanded main piece 5 and IAK question 3 under 3a)	In general, when this management measure expires, other preventive management measures must take its place.
The influence of piping materials (see in detail Chapter 6 and IAK Question 3 under 4a)	The distinction between new buildings/renovation and existing buildings plays a role in efficiency and feasibility. In new/renovated buildings it is easier to prescribe and therefore apply new materials than to replace pipe materials in existing buildings.
The table in 5.2, of Annex 2 of the Legionella Prevention Regulations lacks important risk factors.	Especially with the new requirements around material use, practicability is a concern.

What is the best instrument?

Which tool is best varies by measure.

1. *Only target L. pneumophila in most priority settings (see detailed Chapter 7 and IAK question 3 under 1a)*

Briefly, this measure consists of amending the Legionella Prevention Regulations to require only monitoring for *Legionella* pneumophila in priority institutions. There is one exception to this:

- In priority settings where there is a high density of people with severely weakened immune systems, monitoring culturable *Legionella* spp remains functional because these people are also susceptible to less virulent Legionella species.

Our advice is *to explore* this adjustment *further*, paying attention to the following issues:

- *Legality:* whether the European Commission shares our initial assessment that limiting monitoring to *L. pneumophila* fits into national policy discretion.
- *Feasibility:* whether - in line with our limited research - indeed appropriate detection methods specifically for *L. pneumophila* are available and deployable.

2. *The 1-liter rule (see expanded chapter 8 and IAK question 3 under 2a)*

Briefly, this measure includes dropping the exception for the 1-liter rule. Our advice is to implement this change *in the short term* in the *Regulation legionella prevention*. Only in the area of enforceability are there concerns, but this can be remedied with a reasonable entry into force period for existing cases in the transitional law.

3. *Application of heat shock as a preventive management measure (see detailed chapter 3 and IAK question 3 under 2b)*

In brief, this measure amounts to:

a. for collective piping networks of priority institutions, the risk factors with regard to hot tap water are adjusted. These are as follows:
   - There is no longer talk of growth, neutral and die-off as designating a risk factor, but risk factor should be named as risk of presence of culturable *Legionella* and no risk of presence of culturable *Legionella*.
   - For installations with hot water temperatures lower than 60°C at the hot water system, return pipe of the hot water system, mixing unit and tap point, the qualification: 'risk of presence of culturable *Legionella*'.
   - For installations with hot water temperatures higher than 60°C at the hot water system, return pipe of the hot water system, mixing unit and tap point or installations where the standards set in NEN 1006 are reached through reheating, the qualification: 'no risk of the presence of culturable *Legionella*'.

b. The possibility of controlling legionella, if favorable growth conditions exist, by applying thermal disinfection using heat shocks is dropped if the hot water temperature is below 60°C in
the return pipe, at the mixing device or at the tap point. Under these conditions, the passages in the table in section 5.2 in appendix 2 of the Regulation for the prevention of legionella from drinking water and domestic hot water regarding thermal disinfection at certain temperatures and standstill times are also cancelled.

c. Based on the scientific insights regarding thermal disinfection by means of heat shocks, no unequivocal advice can be given about the application of
thermal disinfection as a management measure at sites where there are favorable growth conditions for *Legionella* and where the hot water temperature is ≥ 60°C. Therefore, two different recommendations are proposed here, one of which can be implemented:
   - let the passages in Table 4 of NEN 1006 regarding thermal disinfection by means of heat shocks at certain temperatures and standstill times also lapse for situations in which the hot water temperature ≥ 60°C. After implementation of this advice, monitor intensively what the influence is on the numbers of *Legionella* in the hot water system, mixing unit and / or the water heater.
   or out-tap pipe. If it is observed that legionella numbers are increasing due to the lapse of this measure, it is recommended that the lapsed passages be reinstated; or
   - For the time being, maintain the passages in Table 4 of NEN 1006 regarding thermal disinfection by means of heat shocks at certain temperatures and standing times if the hot water temperature is ≥ 60°C. At the same time, investigate how successful this control measure is in priority buildings where it is applied.
   Based on the results of the study, it will be possible to then decide whether the measure can be maintained, should be modified, or should be dropped.

Our advice is to implement these amendments under a and b in *the short term* in the *Legionella Prevention Regulations* and to work within the NEN standards sub-committee towards an amendment of this measure in NEN 1006. In the long term, attention must be paid to the availability of other control measures (see IAC question 7). In addition, we recommend that a choice be made between the two recommendations under c and that an experimental provision be considered for this.

4. *Preventive flushing of drinking water and hot tap water systems (see in detail chapter 5 and IAK question 3 under 3a)*

In short, this measure means that preventive flushing of aerosol-forming taps in drinking water and hot water systems will no longer be recognized as a management measure. Our advice is to conduct *further research*. This is because while it is currently unproven that flushing *does* work, it is also unproven that flushing *does not* work. In this
further investigation, the following aspects should become clear:

- A flushing obligation applies to the water quality in the pipes. This flushing obligation will continue to exist even if the flushing obligation for the prevention of *Legionella* is dropped. It must therefore be made clear whether dropping the flushing measure for the prevention of *Legionella* contamination will lead to lower implementation costs in practice.
- Whether there are situations in which the flushing requirement results in
  a burden on priority institutions that is no longer commensurate with its effectiveness.
- Whether there should be an experimental provision that would allow priority institutions to be temporarily exempted from the flushing requirement in order to assess its effects.
- Which management measures are a more effective alternative for the flushing requirement.

5. *The influence of piping materials (see in detail Chapter 6 and IAK question 3 under 4a)*

This measure firstly amounts to including in the regulations a requirement that that the biomass production potential (BPP) (determined with the BPP-
method as described in NEN-EN 16421:2014) of piping materials to be applied in new construction and/or renovation of tap water systems of priority buildings shall not exceed 400 pg ATP/cm2.

Our advice is to implement this new requirement for pipe materials to be used in *the short term*. This requires an amendment to the *Regulations on Materials and Chemicals for Drinking Water and Hot Water Supplies.* Three points of attention apply:

- There are pipe materials that are produced in other EU member states. It is important that new requirements for pipe materials are compatible with rules that apply within the EU or between member states.
- Priority institutions are not a specific category in the *Regulations on Materials and Chemicals for Drinking and Hot Water Supplies.* The same applies to the Building Act 2012. When including this requirement for new construction/renovation in priority institutions, this may create additional legal concerns.
- In the long term, a regulation for existing construction could be considered, for example with a very long transition period.

6. *The table in 5.2, of Annex 2 of the Legionella Prevention Regulations lacks important risk factors.*

In short, this measure involves replacing the table in 5.2 of Annex 2 of the Legionella Prevention Regulations with a new table, which prescribes that the risk analysis must also consider the use of materials and the taps used. Our advice is to implement this measure *in the short term.*
period. During the consultation on the regulation, it is important to give extra attention to the concerns regarding the effectiveness and practicability of the risk factor about material use. It is expected that these concerns can be addressed with a reasonable entry into force period for existing cases in the transitional law.

10.2.3 IAK question 7: what are the implications?

Consequence I: the effectiveness of some frequently used management techniques (flushing, thermal disinfection) is no longer certain based on scientific insights. Other management techniques may come into play to compensate for this.

requirements for the implementation of legionella prevention in order to thereby strengthen the effectiveness of the implementation of legionella prevention in drinking water and hot water systems. The use of the most effective management techniques is a prerequisite for strengthening the effectiveness of the implementation of legionella prevention.
At the same time, an important consequence of the above analysis is that the effectiveness of some commonly used management techniques (flushing, thermal disinfection) is no longer established based on scientific evidence.

There are currently approved available other techniques that may be more effective than the management techniques listed in the regulations. Based on a thorough risk analysis, it can be investigated whether these management techniques are more effective than the management techniques whose effectiveness is no longer established based on this report.

The overview below, prepared by Envaqua, identifies a number of other management techniques:

Techniques	Evidence			Pilots
	KIWA BRL K14010.1	KIWA BRL K14010.2	Ctgb authorization	reduction of hot water /flushing frequency
UVC2	yes	n/a.	n/a.	n/a.
Ultrafiltrate/microfiltration (Point of entry & Point of use) 8	yes	n/a.	n/a.	n/a.
Pasteurization1	yes	n/a.	n/a.	n/a.
AOT+1	yes	n/a.	yes	n/a.
Anodic oxidation1	n/a.	yes	yes	no
Copper/silver ionization2	n/a.	yes	yes	yes

Consequence II: for the system manager, the proposed leads to a process of amending two ministerial regulations and conducting follow-up research.

Based on this study, our proposal is to amend some provisions in the Regulation on Legionella Prevention and the Regulation on Materials and Chemicals in Drinking Water and Hot Water Supply. From the point of view of legislative technique and procedures, this is relatively simple. Of course, the interplay of interests associated with amending these regulations may complicate the ultimate realization of this amendment.
Furthermore, it is suggested twice that further research be done. If this proposal is followed, it is up to the system leader to conduct this research.

Impact III: For implementers and supervisors (see IAK question 2), the proposals lead to adjustments to risk analysis and the application of management measures.

For implementers and supervisors, the consequences consist of adjusting risk analysis and applying control measures. These adjustments require for owners of the systems investments, which, due to reasonable entry into force dates, can be spread out over several years. The advantage is that after the adjustments to the risk analysis and management measures have been implemented, legionella prevention becomes more effective. This means that the risk of legionella infections in the priority institutions is decreasing. For the other implementing parties and supervisors, the changes mean that work processes and documents need to be adjusted.

Consequence IV: for stakeholders, the risk of legionella contamination decreases.

Ultimately, the goal of regulation is to prevent people from becoming ill or dying as a result of Legionella infections. To achieve that goal, regulations must set the most effective requirements. Based on the latest scientific evidence, it can be expected that following the six proposals, which are under IAK question 6 are listed, leads to a strengthening of the effectiveness of the implementation of legionella prevention in drinking water and hot water systems. This results in reducing the risk of legionella infection for stakeholders, especially the vulnerable in priority settings.

10.3 Answering central question and sub-questions

Based on the above line of reasoning, we can answer three questions posed in the questionnaire. To avoid unnecessary duplication, the table below indicates where the answers to these questions can be found.

	QuestionFind
Based on current scientific evidence, what legal requirements in drinking water regulations should be modified, and in what way?	Paragraphs 7 of chapters 39 and 10.2.1 and 10.2.2
What current scientific findings regarding legionella prevention in drinking water systems warrant modification of existing regulations and why?	Paragraphs 5 of chapters 39 and 10.1.3
How can the existing regulations on legionella prevention in drinking water systems be substantively modified?	Paragraphs 7 of chapters 39 and 10.2.1 and 10.2.2

That leaves two questions that require their own independent answers.

What are strengths and weaknesses in theory and practice in current regulations and why?
The strength of the current regulatory environment is evident from the above analysis. The legislative framework for legionella prevention in drinking water and hot water systems consistently follows the framing principles for legislation (see Chapter 2.2). This results in the detailed rules on which the scientific analysis will have the most effect, are housed in ministerial regulations. These regulations are, in a legislative sense, easy to amend. This makes it possible to respond quickly to new scientific insights.

During the study, three weaknesses emerged in theory and in practice.

1. *Regulatory fragmentation*
   The legislative framework for legionella prevention in drinking water and hot tap water systems is certainly not the only legislative framework that focuses on legionella prevention. In chapter 2.2 we outlined other places where legionella prevention is referred to in laws and regulations. These fragmentation has a number of unpleasant consequences. First of all, it leads to a lack of clarity among those implementing the legionella policy. It leads to these parties playing it safe and doing more than is strictly necessary based on the regulations. Second, this fragmentation of regulations also leads to a fragmentation of supervision. Third, some potential sources of contamination are not covered by the regulations.

   This latter consequence may lead to systems falling between the lines that are not available from health perspective do pose a legionella risk and for which legionella prevention is therefore desirable. Often these are systems that - based on a question of definition – are not part of a tap water system but are fed from a tap water system. This applies, for example, to whirlpool baths, Jacuzzis or whirlpools in hotels and recreational dwellings. In addition, new developments such as shower systems with recirculation of the shower water can be considered. The legionella risk with these types of shower systems is still unclear, but it is not consistent that such a shower in a priority location does not need to be sampled.

   There are regulations that can be used to set additional rules for each situation (mainly municipal regulations), but this turns out to be complicated in practice and also only increases the fragmentation mentioned above. For the time being, the obvious course of action is to include potentially risky systems that are filled from the tap water system in the mandatory risk analysis based on the Legionella Prevention Regulations.

2. *Complexity of scope*
   The legislative framework for legionella prevention in drinking water and hot tap water systems, while convenient in design, is complex in scope. Article 35 of the Drinking Water Decree is particularly noteworthy. This complexity is explainable: *Legionella* occurs in different types of buildings and installations and this requires a clever passage on scope. During our investigation it emerged that there are regularly institutions that do not understand this scope provision properly and therefore make unnecessary efforts. A good example is article 35, paragraph 1, in combination with paragraph 4. It states the following:
   1. *This chapter applies to the owner of a collective water supply or collective piping system to which taps as referred to in subsection 4 are directly or indirectly connected (...).*
   4. *The taps referred to in the opening words of subsection 1 shall be:*
      a. *taps with a shower or other appendage that allows water to be sprayed or fogged;*
      b. *taps used, temporarily or otherwise, to connect a shower, other appendage or device capable of spraying or fogging water;*
      c. *taps that the owner reasonably knows or suspects will be used, temporarily or otherwise, to connect a shower, other appendage or device capable of spraying or fogging water;*
      d. *all taps in an institution as referred to in subsection 1(a), to the extent that it is a department is hematology or oncology, or transplants are performed there or patients with chronic lung disease or disorders of the immune system reside there.*

In other words, for most priority institutions the chapter in the Drinking Water Decree on legionella prevention and the underlying regulations apply only to taps with a shower or other fittings that allow water to be sprayed or fogged. During the course of the study, our impression emerged that many priority institutions do not have this restriction to aerosol-forming taps in view at all.

3. *The use of NEN1006*
   Although there is certainly something to be said for the use of NEN1006, our research also reveals a weakness. At the moment that NEN1006 contains a rule that is not in line with the latest scientific insights (for example, about the application of heat shocks), it is not possible for the government to easily modify this rule. This requires the agreement of NEN standards sub-committee.

What adjustments in the implementation practice of legionella prevention in drinking water systems should be made - apart from adjustment of laws and regulations? In what way can this be shaped?

During the study, we came up with a number of possible adjustments to implementation practices that could strengthen the effectiveness of implementing legionella prevention in drinking water and hot water systems.

1. *Collaboration supervisors*
   During the investigation we received signals that some institutions, due to differences in approach by supervisors, do not know where they stand and what is asked of them. Within the legionella prevention in drinking water systems, this has been solved by giving the drinking water companies an important role in carrying out inspections in both priority and non-priority settings. At the same time, for legionella prevention within other systems, supervision is vested in different regulators. By sharing information or carrying out joint inspections, for example, supervision can be improved and standardized. This is also more manageable and predictable for those under supervision.

2. *Better use of big data*
   In the past twenty years of legionella prevention in tap water systems, a multitude of (analysis) data has been collected by plant operators, laboratories, inspectors, enforcers and consultants. In practice, the data exchange between these parties is generally low. This appears to be a missed opportunity, because this data could be used to learn more from practical situations and particularly about the risk factors for legionella growth in tap water systems and about the effectiveness of legionella management. This is also important precisely because regulations can be further improved and tightened based on this. One of the interviewed respondents indicated that it had already made a start on performing big data analyses on analysis results linked to the management and characteristics of tap water systems.
   in buildings. Within the limits of the regulations on data exchange and market regulation, it is possible for the Ministry of IenW to stimulate this exchange of information and use it to monitor the effects of the policy.

3. *Clean design label*
   The finish (roughness) and structural details (seams, corners, cavities) of pipe materials, mixers, shower hoses, shower heads and fittings in the tap water system sometimes explain (rapid) recontamination in tap water systems. In view of climate change and rising temperatures, one of the interviewed respondents pointed out the importance of preparing for a more critical situation with regard to legionella prevention in tap water in the future. This requires that tap water systems be designed "more tightly". The development of a "clean design label" could help in this respect. A "clean design label" would allow manufacturers to qualify their products for use in priority settings such as hospitals and health care institutions. This does not directly involve the thought of regulatory requirements but rather a voluntary "clean design label" that would allow manufacturers to differentiate themselves from the competition.

ANNEX 1

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APPENDIX 2

Evaluation Framework

SubjectAspect	Elaboration		Research	methodR
Problem analysis and goal formulation Come up with supported problem analysis and objective. Answering IAK questions 1 through 4.	Legislation	• How is the legislative and regulatory framework for the prevention of Legionella in drinking water systems structured? (including the Drinking Water Act, Drinking Water Decree, Regulation on the prevention of Legionella in drinking water and warm tap water) • What are strengths and weaknesses in current laws and regulations? Why?	Desk research, interviews, collision test (I)	Analysis legislative framework of approx. 3 A4 by Berenschot, supplementary role KWR
	Forces	• What is the policy environment around Legionella? • What organizations and parties are involved in the prevention of Legionella in drinking water systems? • Who has what role and responsibility? What are the interests and needs of each party? • What interrelationships exist between involved organizations and parties?	Desk research, interviews, collision test (I)	Force field analysis of approx. 23 A4 by KWR, additional role Berenschot
	Science	• What new scientific understanding of Legionella has been gained in recent years? (This section focuses at least on hot water temperature, Legionella pneumophila versus nonpneumophila, cold water versus hot water systems, and the one-liter rule, but can be expanded as a result of discussions with the guidance committee and experts) • What bottlenecks in laws and regulations and/or implementation practices surrounding legi onella prevention in drinking water systems can be identified based on new scientific insights? • What suggestions can be made for amending laws and regulations and/or implementation practices?	Literature research interviews, collision test (II)	Description of scientific insights by KWR
	Intermediate problem definition and objective: We are preparing an interim product in which we set out the pro blem definition and objective based on the information collected. This interim product serves as a factual basis and an elaboration of the tracks that will be further developed in the sequel. It is coordinated with the client and the supervisory committee. By problem definition and objective we mean the following: • *Problem definition*: On the basis of analysis of legislation, the field of influence and science, a description of the current factual situation, how it is valued and why. In describing the problem we make a distinction between the scientific facts (relationships between causes and effects) on the one hand and the appreciation of those facts on the other. The facts are the same for everyone, the appreciation of them depends on the interests that people have and the values that they hold. And these can differ from party to party. • *Objective*: What objective can be formulated to address the problem identified solve?		Analysis research team	Bundling of analysis of legislation, field of forces and scientific insights. Description of problem definition and objective where possible in graphic form in approx. 3 A4 by Berenschot, supplementing The role of KWR
Government intervention and tools IAK question 5, 6 **Effects** IAK Question 7		• What changes need to take place to achieve the objective? • What tools can be used - apart from amending laws and regulations? • Is there a task for the central government? What is role/involvement of other parties?	Analysis on der research team using roadmap instrument selection, collision test (III)	Analysis of instru ments choice and government intervention by Berenschot, reviewing/complementing role of KWR
		• What is the impact of potential interventions on the prevention of Legionella in drinking water systems? • What are the implications of potential interventions for stakeholders (including feasibility, enforceability)?	Analysis on der research team, impact test (III)	Analysis of impacts by Berenschot, reviewing/complementing role of KWR

SubjectAspect	Elaboration	Research	methodR
Conclusion and Recommendations.	• Based on current scientific knowledge, which legal requirements in drinking water regulations should be adjusted, and in what way? • What current scientific insights regarding legionella re vention in drinking water systems warrant modification of existing regulations and why? • What are strengths and weaknesses in theory and practice in the current rulebook and why? • How can the existing regulations on legionella prevention in drinking water systems be substantively modified? • What adjustments in the implementation practice of legionella prevention in drinking water systems - apart from adjustment of laws and regulations - should be made? How can this be done?	Analysis under search team	Answering the central question and 3 subquestions. Recommendations regarding amendments to laws and regulations and/or implementation practices by Berenschot and KWR
	Draft and final report On the basis of the information collected on the instruments, government intervention and consequences, and the third collision test, we will analyse the results and produce a draft report. We present this to the client and the supervisory committee. After receiving comments, we prepare the final report.		

ANNEX 3

Overview of interviewees, members of the supervisory committee and participants in the LOPL impact study

This report is the result of research conducted by Berenschot and KWR Water Research Institute on behalf of the Ministry of IenW. The contents and quality of the report are the explicit responsibility of Berenschot and KWR Water Research Institute. The individuals listed below contributed to the study in various roles, but are not responsible for its content or quality.

	IntervieweesOrganization
	RIVM
Anne Laming, Frits Mul and Martien	JanssenVerenigingGehandicaptenzorg
NederlandDanny SchoonrokVan	Hoften Installatietechniek
	ILT
Hans van WolferenVan	Wolferen Advies
Irene van VeelenISSO	
Kevin KantersHydroscope/Envaqua	GGD Haaglanden
Monique BastmeijerStichting	Veterans Disease
Oscar NuijtenEdu4Install	
Sjoerd EuserStreaklab	Haarlem
	Ministry of IenW
Will SchefferTVVL	Expert Group Sanitary Techniques

Members	supervisory committeeOrganization
Egbert LeitingEnvaqua	
Eric van der	
	BlomTechniekNederlandILT
	ILT
Monique BastmeijerStichting	Veterans Disease
Onno	LeeverISSO
Oscar NuijtenEdu4Install	
	RIVM
Rick LangenVEWIN	
	State Property Agency Ministry of IenW
Ans	
	VersteeghStichtingVeteran's DiseaseMinistry of Health, Welfare and Sport
Jeroen van den	HeuvelHISWARECRON

Participants crash test	LOPLOrganisation
Brenda van RijnLOPL	
	North Brabant Environment Service
Elisa van	KenhoveUniversity ofGhent
Eric van der	BlomTechnologyNetherlands
Frank OesterholtKWR	
Ger ArdonLOPL	
Kevin KantersEnvaqua	
Leo	BikkerAdvisor
Monique BastmeijerStichting	Veterans Disease RIVM
Raymond van der	HeydenNVTG
Simcha GeleynseKIWA	
	State Property Administration
Walter van der ScheeTVVL	

ANNEX 4

Increased yield: bottlenecks experienced by the field

During the interviews, respondents were asked what other topics they felt were relevant in the context of current legionella legislation and were not included in the current study. This chapter contains a point-by-point listing of those topics:

- More room should be created in the regulations for the application of other management concepts in non-priority institutions that do house people at increased risk (e.g. housing complexes for the elderly). This is also in line with government policy of allowing the elderly to live at home for as long as possible.
- Towards the future, more attention should be come for 'clean design' of tap water systems, for example, linked to a clean design label. If the average temperature is going to rise under the influence of climate change, the importance of constructive details will only increase.
- There is a big difference in intensity between the limited risk analysis versus the extensive risk analysis as included in appendix 2 of the Legionella prevention regulation (regulation 1.2.1 b and c respectively; note: *the terminology limited and extensive themselves are not used in the Regulation)*. Regulation 1.2.1 b (note: *corresponds to limited RA)* actually makes it appear that you are done, so to speak, if you place a filter on all aerosol forming taps where there are also many snags, while otherwise you end up in a whole complex of control measures.
- The disability sector has very different concerns than the (technical) issues mentioned here. From the sector's point of view, they have a different interest in mind. For them the main question is how the rules can be organized in such a way that it is in the interest of their residents. Currently, the regulations result in performing unnecessary work that costs a lot of time and money, but which in practice is not at all in the interest of the residents. So that's separate from the technology and whether effective legionella management can be performed.
- With the huge data set of analysis data we have in the Netherlands, we can theoretically learn a lot about the risk factors for growth of *Legionella* and about the effectiveness of legionella management. Hydroscope and other companies are already building up a dataset based on the legionella analyses they perform and using it to perform big-data analyses using smart algorithms. Such an analysis would require some time, estimated to be about five years. Based on that analysis, you would be able to adapt the legislation more specifically after five years. A tricky point here is that laboratories cannot simply share customer data.
- The possibilities for data exchange between parties involved in the regulations (installation managers, laboratories, inspection, advisors) are far too underused. There is a wealth of data that we could use to learn more together from practical situations, precisely in order to improve the regulations on that basis. The enforcement campaigns by ILT provide a lot of information on shortcomings in practice, but these are not widely fed back. The current legislation does not currently include such feedbacks and learning effects.
- From a health perspective, it is important to note that there are systems that are not under regulations, think of hot tubs in hotels and recreational facilities and, for example, recycle showers. Based on a matter of definition, these are systems that are linked to a tap water system and therefore do not fall under the Regulation on Legionella Prevention in Drinking Water and Hot Tap Water, but can pose a risk.
- Other management techniques and their effectiveness. The RIVM study regarding alternative management could use an update. What are the latest insights scientifically on that point? Perhaps there are conceivable situations where the thermal management concept is not the best solution.
- Role of VBNC status in sampling after cleaning and disinfection. Is the culture method suitable? Important because practice shows that often re-infection occurs quickly.
- Difference in degree of aerosol formation between taps.
- Increased understanding of dose-effect relationship of *Legionella*.
- Lack of clarity on required security when connecting heat pumps.
- Knowledge level of installers in practice still needs improvement. It would be useful if the little *Legionella,* a pocket guide for installers, were to be updated, so that mechanics are better informed about this again.

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