Detection of *Legionella pneumophila* and *Pseudomonas aeruginosa* and biofilm experiments in four different cooling towers (construction and biocide treatment) and evaluation of IDEXX most probable number methods in industrial water samples

Dissertation

zur

Erlangung des Doktorgrades (Dr. rer. nat.)

der Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Kirsten Spies (geb. Braun)

aus

Köln

Bonn, 2021

Angefertigt mit Genehmigung der

Mathematisch-Naturwissenschaftlichen Fakultät der

Rheinischen Friedrich-Wilhelms-Universität Bonn

Erstgutachter: Herr Prof. Dr. Dr. Martin Exner

Zweitgutachter: Herr Prof. Dr. Uwe Deppenmeier

Tag der Promotion: 27.09.2021

Erscheinungsjahr: 2021

„Die Kunst zu heilen kann viel Leid lindern, doch schöner ist die Kunst, die es versteht, die Leiden am Entstehen schon zu hindern“

Max von Pettenkofer (1818 – 1901)

Meinen Eltern

Abstract

The responsible maintenance of evaporative cooling systems is an essential element in reducing the risk of the environmental dispersion of legionellae and Legionnaires' disease outbreaks. In Germany, the 42nd Ordinance Implementing the Federal Immission Control Act (42nd BImSchV) rules in accordance with the German Cooling Tower Code of Practice VDI 2047-2 the cooling tower water monitoring of *Legionella* spp., heterotrophic plate counts (HPC) and *P. aeruginosa* by ISO methods. The studies in this thesis aimed to improve microbial cooling tower monitoring and surveillance.

For an enhanced surveillance of evaporative cooling systems, reliable prediction tools for microbial concentrations in the cooling system would be very advantageous. A prediction tool was designed using correlation analyses of retrospective microbiological and the process parameter data water temperature or redox potential. The tool requires a cooling tower specific testing for its suitability to promote the early initiation of measures. Thus, an increase in biofilm might be preventable at an early stage. Another simple risk factor calculation tool has been created indicating the microbial status of the cooling system. The risk factor calculation based on *Legionella* concentrations or combined *Legionella* concentrations and HPC of previous sample results. Depending on the level of the cooling tower-specific risk factor, graduated measures to irregularities in the operation mode can be implemented cooling tower specific.

The ISO methods for the detection of microorganisms in cooling water samples apply agar plates and their evaluation is often complicated by the high amount of accompanying microorganisms. Alternative methods for a cooling tower monitoring are provided by the IDEXX most probable number methods Legiolert™/Quanti-Tray® for the detection of *L. pneumophila* and Pseudalert™/Quanti-Tray® for the detection of *P. aeruginosa*. Comparative testing of ISO and IDEXX methods in numerous industrial samples demonstrated that the IDEXX methods are easier in handling and reading of results and offer a very good suitability for the detection of these parameters in cooling tower water samples.

The detection of HPC by the ISO method is carried out at two incubation temperatures. Due to the high amounts of microorganisms in cooling water, counting the colony forming units is laborious and the production of dilution series is time and material consuming. Therefore, the HPC results of 2,868 industrial samples from the Laboratory for Technical Hygiene were analysed to determine the feasibility of incubation at only one temperature. The analysis showed that the use of one temperature is in principle sufficient to trace biofilm formation in the cooling system without loss of information.

To evaluate the influence of the construction material and biocide on the biofilm formation microbial growth on stainless steel and polyethylene plates and planktonic microorganisms were analysed in four closely located cooling towers sourced by the same make-up water during a ten-month observation period. Results of this study showed that the cooling towers differed in their microbial composition. Statistically significant differences in biofilm formation on the different plates materials were not verified. This indicates that the biocide and not the construction material seems to have the major impact on the biofilm formation. The lowest sessile and planktonic microbial concentrations were observed in cooling towers treated with chlorine dioxide and ozone. In accordance with the literature, the initial microbial community persisted over a long period as long as the biocide was able to limit biofilm growth. Increased visually assessed biofilm thickness on the long-term plates was associated with significantly different short- and long-term concentrations of sessile microorganisms.

Seasonal dynamics of *Legionella* and HPC in the four cooling towers were analysed by using retrospective data. Each cooling tower showed specific dynamics, but always *Legionella* tended to be highest in late summer/autumn and HPC in midsummer. Within four weeks, heterotrophic microorganisms cultivable under ISO conditions reached concentrations up to 10^8 cfu/cm^2. Planktonic *Legionella* increased to an objectionable level within six weeks under deactivated biocide treatment in a cooling tower of always low microbial concentrations. Based on both latter facts, the regular four-week inspection interval of the 42nd BImSchV is considered appropriate with regard to the protection of public health.

1 Introduction 1
1.1 The role of legionellae and biofilms in cooling towers 1
1.1.1 *Legionella* spp. 2
1.1.1.1 Microbiology 2
1.1.1.2 Transmission 3
1.1.1.3 Incidence, virulence and symptoms 5
1.1.1.4 Risk factors 6
1.1.2 Biofilm formation in cooling towers 7
1.2 German regulations for evaporative cooling systems 14
1.3 Microbiological detection methods in cooling water samples 15
1.3.1 Detection of *Legionella* spp. and *Legionella pneumophila* 16
1.3.1.1 UBA recommended ISO 11731 procedures 17
1.3.1.2 Legiolert 17
1.3.2 Detection of heterotrophic plate counts 17
1.3.3 Detection of *Pseudomonas aeruginosa* 18
1.3.3.1 DIN EN ISO 16266:2008 19
1.3.3.2 Pseudalert 19
1.4 The four investigated cooling towers of this study 19
1.4.1 Cooling Tower 1 21
1.4.2 Cooling Tower 2 22
1.4.3 Cooling Tower 3 24
1.4.4 Cooling Tower 4 25
1.5 Aim of this dissertation 27
2 Material and Methods 28
2.1 List of devices 28
2.2 Chemicals, solutions and nutrient media 29
2.3 Plates holding unit for the biofilm experiments 31
2.4 Sampling 32
2.4.1 Water samples of the comparative analysis of ISO and Legiolert 32
2.4.2 Water samples and swabs of the biofilm experiments 32
2.4.2.1 Water samples 32
2.4.2.2 Observation period 32
2.4.2.3 Removal of plates 33
2.4.2.4 Biofilm swabs 33
2.5 Microbiological Methods 34
2.5.1 Detection of *Legionella* and *L. pneumophila* 37
2.5.1.1 Detection of *Legionella* spp. by procedures of the ISO method 37
2.5.1.1.1 ISO procedures according to the UBA recommendation 38
2.5.1.1.2 Biofilm experiments in four cooling towers 40
2.5.1.1.2.1 Water samples 40

2.5.1.1.2.2 Biofilm suspensions 40
2.5.1.1.3 “Bonn method” performed for results of the retrospective analysis and surveillance in one cooling tower under deactivated biocide treatment conditions 41
2.5.1.2 Detection of *L. pneumophila* by Legiolert 42
2.5.1.3 *Legionella* species and serotype testing via latex agglutination 44
2.5.2 Detection of heterotrophic plate counts 44
2.5.3 Detection of *P. aeruginosa* 45
2.5.3.1 ISO 16266 method 46
2.5.3.2 Pseudalert 46
2.5.4 Confirmation of isolate identity of positive Quanti-Tray wells 47
2.5.5 Counting rules of the agar plated Biofilm suspension 47
2.6 Data analysis 48
2.6.1 Retrospective data analysis 48
2.6.2 Evaluation of measurement uncertainty 50
2.6.3 Comparative data analysis 50
2.6.3.1 McNemar’s test 50
2.6.3.2 Comparative analysis according to ISO 17994 50
2.6.3.3 Significance analyses 51
3 Results 52
3.1 Comparative analysis of Legiolert and the ISO method 52
3.2 Retrospective analyses of microbial concentrations in cooling tower samples 61
3.2.1 Retrospective analysis of process parameters and microbiological concentrations in four cooling towers 61
3.2.1.1 Cooling Tower 1 64
3.2.1.2 Cooling Tower 2 69
3.2.1.3 Cooling Tower 3 75
3.2.1.4 Cooling Tower 4 80
3.2.1.5 Distribution of *Legionella* concentrations according to 42nd BImSchV in the four cooling towers 85
3.2.2 Seasonal dynamics in the four cooling towers 87
3.2.3 Comparison of heterotrophic plate counts at the incubation temperatures of 22 and 36 °C detected with the ISO method 90
3.3 Biofilm formation in four different cooling towers 95
3.3.1 Visual assessment of the four cooling towers 95
3.3.2 Microbial growth on the stainless steel and polyethylene plates and in water samples 97
3.3.2.1 Cooling Tower 1 98
3.3.2.2 Cooling Tower 2 104
3.3.2.3 Cooling Tower 3 112
3.3.2.4 Cooling Tower 4 121

3.3.3 Microbiological composition of the water samples from the four cooling towers 130
3.3.3.1 Microbial concentrations in the water samples of the four cooling towers 130
3.3.3.2 Exceedance of 42nd BImSchV test and action values and risk assessment 136
3.3.4 Microbial composition of the biofilm grown on the stainless steel and polyethylene plates in the four cooling towers 141
3.3.5 Comparative analyses of ISO and IDEXX methods in 40 cooling tower water samples 148
3.3.5.1 Comparative analysis of Legiolert and the UBA recommended ISO procedures 148
3.3.5.2 Comparative analysis of Pseudalert and ISO 16266 for the detection of *P. aeruginosa* 153
3.4 Biofilm formation in a cooling tower under deactivated biocide treatment conditions 155
3.4.1 Redox potential curve before, during and after deactivated biocide treatment 156
3.4.2 Microbial concentrations in water samples 157
3.4.3 Microbial composition of the biofilms grown on stainless steel and polyethylene plates 159
3.4.4 Microbial distribution in water samples and in biofilms grown on plates 161
4 Discussion 164
4.1 Applicability of methods for the detection of microorganisms in cooling tower water samples 164
4.1.1 Applicability of Legiolert™/Quanti-Tray® for the detection of *Legionella pneumophila* in cooling tower water 164
4.1.2 Applicability of Pseudalert™/Quanti-Tray 2000 ® for the detection of *P. aeruginosa* in cooling tower water 168
4.1.3 Applicability of the ISO method for the detection of heterotrophic plate counts in cooling tower water 169
4.2 Assessment of the retrospective analyses for the prediction of legionellae concentrations and heterotrophic plate counts 172
4.3 Microbiological planktonic concentrations and biofilm formation in the four cooling towers 173
4.3.1 Similarities and differences of the four investigated cooling towers 174
4.3.2 Seasonal microbiological dynamics 179
4.3.3 Influence of the make-up water 180
4.3.4 *Legionella* in biofilms and cooling tower risk management 180
4.3.5 Comparison of free-living planktonic and biofilm-associated bacteria 185
5 Conclusions 189
6 Bibliography 190
7 Data Annex 200

Abbreviation	Meaning
0.5 log level-rule	In the Laboratory for Technical Hygiene, this rule is used as an indicator whether microbiological results can be regarded as or differ significantly. By definition, results are equal if the difference of the log-transformed results is less than 0.5. The rule is applied for results of the same method from the same sample tested in replicates or for results of the same sampling site tested at different times.
42nd BImSchV	42. Bundesimmisionsschutzverordnung (ger.): German 42nd Ordinance Implementing the Federal Immission Control Act
BCYE	Buffered Charcoal Yeast Extract agar
BCYE+AB	Buffered charcoal Yeast Extract agar plus antibiotics (Polymyxin B, Pimaricin, Cefazolin)
cfu	Colony forming units
CT	Cooling tower
DAkkS	Deutsche Akkreditierungsstelle (ger.): German Accreditation Body
DEV	Deutsche Einheitsverfahren (ger.): German standard procedures
GVPC	Glycin Vancomycin Polymycin B Cycloheximide
HPC	Heterotrophic plate counts
ISO	International Organization for Standardization
KaVKA	Katasteramt für Verdunstungskühlanlagen (ger.): land registry office for evaporative cooling systems
LD	Legionnaires' disease
Lp	*Legionella pneumophila*
Lp1	*Legionella pneumophila* serogroup 1
Lp2-14	*Legionella pneumophila* serogroup range 2 to 14
Lsp	*Legionella* spp.
mpn	Most probable number
MU	Measurement uncertainty
n	not applicable due to too high concentration of interfering bacteria
NBT	No biocide treatment
PA	*Pseudomonas aeruginosa*
PE	Polyethylene
Proc.	procedure/s
rec.	recommended
RefVal	Reference value for heterotrophic plate counts according to 42. BImSchV
SG	Serogroup
SS	Stainless steel
TNTC	Too numerous to count (exceeding the upper quantification level)
UBA	Umweltbundesamt (ger.): Federal Environment Agency
UQL	Upper quantification limit
VAH	Verbund für Hygiene (ger.): associaion for Hygiene
xx_LI	LI, long interval (in combination with PE or SS)
xx_SI	SI, short interval (in combination with PE or SS)

Units are listed according to the current IUPAC nomenclature.

Figure 1: Life cycle, reservoir and mode of transmission of *Legionella*. 4
Figure 2: Schematic illustration of a wet cooling tower with open circuit. 7
Figure 3: Biofilm formation. 9
Figure 4: Cooling Tower 1. 21
Figure 5: Cooling Tower 2. 23
Figure 6: Cooling Tower 3. 25
Figure 7: Cooling Tower 4. 26
Figure 8: Plates-holding unit. 31
Figure 9: Distribution of the UBA recommended ISO procedures for the calculation of the final result. 53
Figure 10: Location of the “confidence intervals” from comparative analyses according to ISO 17994 of paired data from Legiolert and ISO *L. pneumophila* results. 55
Figure 11: Location of the confidence intervals of relative differences [%] from comparative analyses according to ISO 17994 of paired data from Legiolert and ISO 11731 *Legionella* spp. results. 57
Figure 12: Measurement uncertainty (MU) of ISO and Legiolert counts. 60
Figure 13: CT1 - *Legionella* spp. concentrations from 2012 to 2018. 65
Figure 14: CT1 – Distribution of *Legionella* strains in 84 water samples from 2012 to 2018. 65
Figure 15: CT1 - HPC from 2017 to 2019. 65
Figure 16: CT1 - Boxplots of the different data sets. 66
Figure 17: CT1 - Regression lines of the microbiological concentrations and modified data derived from the redox potential data. 67
Figure 18: CT1 - Regression line of the log10 HPC at 22 °C and the minimum water temperature seven days before sampling. 68
Figure 19: CT2 - *Legionella* spp. concentrations from 2012 to 2018. 70
Figure 20: CT2 – Distribution of *Legionella* strains in 84 samples from 2012 to 2018. 70
Figure 21: CT2 - HPC from 2017 to 2019. 70
Figure 22: CT2 - Boxplots of the different data sets. 71
Figure 23: CT2 - Regression lines of microbiological concentrations and modified data derived from the redox potential data. 73
Figure 24: CT2 - Regression lines of microbiological concentrations and modified data derived from the water temperature data. 74
Figure 25: CT3 - *Legionella* spp. concentrations from 2012 to 2018. 76
Figure 26: CT3 – Distribution of *Legionella* strains in 84 samples from 2012 to 2018. 76
Figure 27: CT3 - HPC concentrations from 2017 to 2019. 76
Figure 28: CT3 - Boxplots of the different data sets. 77
Figure 29: CT3 - Regression lines of microbiological concentrations and modified data derived from the redox potential data. 79
Figure 30: CT4 - *Legionella* spp. concentrations from 2012 to 2018. 81
Figure 31: CT4 – Distribution of *Legionella* strains in 84 samples from 2012 to 2018. 81

Figure 32: CT4 - HPC concentrations from 2017 to 2019. 81

Figure 33: CT4 - Boxplots of the different data sets. 82

Figure 34: CT4 - Regression lines of microbiological concentrations and modified data derived from the water temperature potential data 84

Figure 35: Distribution of the *Legionella* concentrations in the four cooling towers from 2012 to 2018 divided according to the test and action values of the 42nd BImSchV.... 85

Figure 36: Seasonal dynamics of *Legionella*. 88

Figure 37: Seasonal dynamics of HPC. 89

Figure 38: Distribution of quantifiable HPC according to the laboratory intern "0.5 log level-rule" 92

Figure 39: Location of the confidence intervals of relative differences from comparative analyses according to ISO 17994 of paired data from HPC at 22 and 36 °C. 94

Figure 40: Plates after removal from Cooling Tower 1. 98

Figure 41: CT1 - Microbial growth on the SS and PE plates and in the water sample. 101

Figure 42: CT1 - Distribution of *Legionella* strains in water samples (ISO method; n = 11) and on the different short-term (n = 10) and long-term (n = 9) plates. 103

Figure 43: Plates after removal from Cooling Tower 2. 105

Figure 44: CT2 - Microbial growth on the SS and PE plates and in the water sample. 109

Figure 45: CT2 - Distribution of *Legionella* strains in water samples (ISO method; n = 11) and on the different short-term (n = 10) and long-term (n = 9) plates. 111

Figure 46: Plates after removal from Cooling Tower 3. 112

Figure 47: CT3 - Microbial growth on the SS and PE plates and in the water samples..... 115

Figure 48: CT3 - Distribution of *Legionella* strains in water samples (ISO method; n = 11) and on the different short-term (n = 10) and long-term (n = 9) plates. 118

Figure 49: Plates after removal from Cooling Tower 4. 121

Figure 50: CT4 - Microbial growth on the SS and PE plates and in the water sample. 125

Figure 51: CT4 - Distribution of *Legionella* strains in water samples (ISO method; n = 11) and on the different short-term (n = 10) and long-term (n = 9) plates. 127

Figure 52: Microbiological composition of the water samples from the four cooling towers. 132

Figure 53: Value ranges of the microbiological parameters in the water samples from the four cooling towers. 133

Figure 54: *Legionella* concentrations in the four cooling towers over the ten-month investigation period divided according to test and action values of the 42nd BImSchV. 137

Figure 55: Cooling Tower specific risk factor calculation. 138

Figure 56: Risk assessment for the four cooling towers based on different observation periods. 139

Figure 57: Microbiologicl composition of the biofilm grown on the stainless steel plates from the four cooling towers. 144

Figure 58: Microbiological composition of the biofilm on the polyethylene plates from the four cooling towers. 145

Figure 59: Boxplots of the microbiological parameters grown in biofilms on the stainless steel plates from the four cooling towers. 146

Figure 60: Boxplots of the microbiological parameters grown in biofilms on the polyethylene plates in the four cooling towers. 147

Figure 61: Location of the confidence intervals from comparative analyses according to ISO 17994 of paired data from Legiolert and *L. pneumophila* or *Legionella* spp. results of modified UBA recommended ISO procedures. 150

Figure 62: Measurement uncertainty of ISO and Legiolert counts of the water samples from the biofilm experiments. 152

Figure 63: Distribution of the modified UBA recommended ISO procedures for the final result calculation. 152

Figure 64: Location of the confidence interval from relative differences of the comparative analysis according to ISO 17994 of paired data from Pseudalert and the ISO method. 154

Figure 65: Measurement uncertainty of Pseudalert and ISO counts. 154

Figure 66: Redox potential before and during deactivation and after reactivation of the biocide treatment. 156

Figure 67: CT1 - Deactivated biocide treatment: microbial composition of the water samples. 158

Figure 68: CT1 - Deactivated biocide treatment: microbial growth on plates. 160

Figure 69: CT1- Deactivated biocide treatment: microbial growth on the stainless steel and polyethylene plates and in the water samples. 162

Figure 70: Risk factor calculation of the latest 40 values. 183

Table 1: Test and action values of the German 42nd Ordinance Implementing the Federal Immission Control Act (42nd BImSchV). 14

Table 2: Fact sheet of the four investigated cooling towers. 20

Table 3: List of devices. 28

Table 4: List of chemicals, solutions and nutrient media. 29

Table 5: Microbiological methods performed in this work. 35

Table 6: Volumes of the microbiological tests of the biofilm suspensions. 36

Table 7: Quantification limits and measurement uncertainty of applied microbiological methods according to UBA and ISO 8199. 36

Table 8: ISO 11731 procedures. 37

Table 9: McNemar's test for max. or best Legiolert vs. ISO *L. pneumophila* counts. 54

Table 10: McNemar's test for max. or best Legiolert vs. ISO *Legionella* spp counts. 54

Table 11: McNemar's test for max. Legiolert vs. ISO *L. pneumophila* (left side of the slash) and *Legionella* spp. (right side of the slash) counts. 54

Table 12: McNemar's test for best Legiolert vs. ISO *L. pneumophila* (left side of the slash) and *Legionella* spp. (right side of the slash) counts. 54

Table 13: Comparative analyses of paired data from Legiolert max. and best results with ISO *L. pneumophila* counts. 55

Table 14: Various comparative analyses according to ISO 17994 of paired data from Legiolert and ISO *Legionella* spp. counts. 56

Table 15: Classification of Legiolert max. and ISO *L. pneumophila* results acc. to 42nd BImSchV. 59

Table 16: Classification of Legiolert best and ISO *L. pneumophila* results acc. to 42nd BImSchV. 59

Table 17: Classification of Legiolert max. and ISO *Legionella* spp. results acc. to 42nd BImSchV. 59

Table 18: Classification of Legiolert best and ISO *Legionella* spp. results acc. to 42nd BImSchV. 59

Table 19: CT1 – Number of outliers of the microbiological data. 67

Table 20: CT1 - Results of the regression analyses of the microbiological and redox potential data. 67

Table 21: CT1 - Results of the regression analyses of the microbiological and water temperature data. 68

Table 22: CT2 – Number of outliers of the microbiological data. 72

Table 23: CT2 - Results of the regression analyses of the microbiological and redox potential data. 73

Table 24: CT2 - Results of the regression analyses of the microbiological and water temperature data. 74

Table 25: CT3 – Number of outliers of the microbiological data. 78

Table 26: CT3 - Results of the regression analyses of the microbiological and redox potential data. 79

Table 27: CT3 - Results of the regression analyses of the microbiological and water temperature data. 80

Table 28: CT4 – Number of outliers of the microbiological data. 83

Table 29: CT4 - Results of the regression analyses of the microbiological and redox potential data. 83

Table 30: CT4 - Results of the regression analyses of the microbiological and water temperature data..... 84

Table 31: *Legionella species* detections in each 84 samples from the four cooling towers. 86

Table 32: HPC results of industrial samples from the Laboratory of Technical Hygiene. 91

Table 33: Distribution of quantifiable HPC at the incubation temperatures of 22 and 36 °C..... 92

Table 34: ISO 17994 comparative analyses for HPC at the incubation temperatures of 22 and 36 °C. 94

Table 35: Visual assessment of the four cooling towers considering the water clarity and the algae growth on internals 96

Table 36: Cooling Tower 1 - Microbial concentrations in water samples. 100

Table 37: Cooling Tower 1 - Microbial concentrations in the biofilms grown on a) stainless steel and b) polyethylene plates. 100

Table 38: Cooling Tower 2 - Microbial concentrations in water samples. 108

Table 39: Cooling Tower 2 - Microbial concentrations in the biofilms grown on a) stainless steel and b) polyethylene plates. 108

Table 40: Cooling Tower 3 - Microbial concentrations in water samples 114

Table 41: Cooling Tower 3 - Microbial concentrations in the biofilms grown on a) stainless steel and b) polyethylene plates. 114

Table 42: CT3 - Distribution of Legionella in water samples (ISO method) and on the different plates. 118

Table 43: Cooling Tower 4 - Microbial concentrations in water samples. 124

Table 44: Cooling Tower 4 - Microbial concentrations in the biofilms grown on a) stainless steel and b) polyethylene plates. 124

Table 45: CT4 - Distribution of *Legionella* in water samples (ISO method) and on the different plates. 127

Table 46: Boxplot values of the microbiological parameters in the water samples from the four cooling towers. 133

Table 47: Boxplot values of the microbiological parameters in the biofilm of the stainless steel plates in the four cooling towers. 146

Table 48: Boxplot values of the microbiological parameters in the biofilm of the polyethylene plates in the four cooling towers. 147

Table 49: McNemar's test of the 40 cooling tower samples for Legiolert max. vs. modified UBA recommended ISO results for *L. pneumophila* and *Legionella* spp. counts. 149

Table 50: Comparative analysis according to ISO 17994 of paired data from Legiolert and *L. pneumophila* and *Legionella* spp. counts from modified UBA recommended ISO procedures. 150

Table 51: Classification of Legiolert max. and ISO *L. pneumophila* results according to 42nd BImSchV. 151

Table 52: Classification of Legiolert best. and ISO *L. pneumophila* results according to 42nd BImSchV. 151

Table 53: Classification of Legiolert max. and ISO *Legionella* spp. results according to 42nd BImSchV. 151

Table 54: Classification of Legiolert best and ISO *Legionella* spp. results according to 42nd BImSchV. 151

Table 55: Mc Nemar's Test of the 40 cooling tower samples for Pseudalert vs. ISO results 153

Table 56: Comparative analysis of paired data from Pseudalert and the ISO method. 154

Table 57: CT1 - Deactivated biocide treatment: microbial concentrations in water samples. 158

Table 58: CT1- Deactivated biocide treatment: microbial concentrations in the biofilms. ... 160

Table 59: Measurement uncertainty of final results exceeding test and action values of the 42nd BImSchV, depending on the test volume. 168

Table 60: Statistical significant differences of growth on short-term and long-term plates for each cooling tower. 177

Table 61: Relation of biofilm-associated and planktonic microorganisms in [%]. 185

1 Introduction

“Verdunstungskühlanlagen können tödlich sein -
Evaporative cooling systems can be lethal” (33)

This statement of Prof. Dr. Dr. Martin Exner concerns evaporative cooling systems, which are not adequately maintained and microbiologically monitored. This is demonstrated by legionellosis outbreaks and epidemics that have been linked to evaporative cooling systems (14, 41, 164, 172). Their "deadly effect" is caused by bacteria of the genus *Legionella*. The water-born Gram-negative bacteria generally find ideal conditions in evaporative cooling systems for reproduction. The temperature range and the nutrients introduced by air inflow offer excellent conditions for the proliferation of bacteria (36, 42, 159). *Legionella* are distributed via aerosols, which are produced during the cooling process of the water (24, 41, 42, 66, 130, 145). In particular, the species *Legionella pneumophila* is the predominant cause for legionellosis and associated with worldwide legionellosis outbreaks caused by cooling towers (2, 12, 24, 31, 37, 38, 41, 47, 49, 50, 54, 58, 59, 66, 68, 83, 85, 87, 101, 112, 113, 120, 124, 133–135, 138, 164, 167, 169). In general, legionellosis can be avoided by prevention including monitoring and surveillance actions followed by eliminating procedures of a potential reservoir (24, 41, 102).

1.1 The role of legionellae and biofilms in cooling towers

Cooling towers display a particular problem since it is assumed that cooling towers account for at least one quarter of all sporadic cases of legionellosis (146).

Between the years 2002 and 2007, 44 outbreaks of Legionnaire’s disease were attributable to wet cooling systems involving 1,175 cases, as reported to the EWGLI annual dataset by eleven collaborating countries (129). The largest legionellosis outbreak in Germany caused by a contaminated cooling tower occurred in Warstein in 2013 with 159 suspected cases. The case-fatality rate of 1 % was low compared with the European mean of 8 % (32), probably because of immediate clinical treatment of symptomatic individuals. Due to forced epidemiological assessment, source tracing, shutting down of potential sources and rapid laboratory testing the source was identified within 20 days after the first legionellosis case and seven days after the outbreak was reported to public health authorities (97).

Insufficient maintenance actions are often the cause of legionellosis outbreaks associated with cooling towers (14, 41, 164, 172). Consequently, some countries have already established registers and guidelines for cooling towers. With the introduction of the German 42nd Ordinance Implementing the Federal Immission Control Act (“42nd BImSchV” (1)) in 2018 and land

registry office for evaporative cooling systems (KaVKA) in 2019, legislation and registration also exist in Germany.

1.1.1 *Legionella* spp.

After a large outbreak of pneumonia among the members of the 58th convention of the American Legion in July 1976 in Philadelphia, a previously unrecognized bacterium was identified as the causative agent by Joseph McDade and Charles Shepard and designated as *Legionella pneumophila* in 1977 (24, 41, 46, 103). The genus and species name refers to the first occurrence and the organ affected (66). An airborne transmission of the bacteria in the lobby area and in the immediate vicinity of the hotel was suspected as the source of infection (46). In this outbreak, 34 of the 221 patients died resulting in a case-fatality rate of 15 % (24).

1.1.1.1 Microbiology

The family Legionellaceae consists of the only genus *Legionella* and represents a monophyletic subgroup within the gamma-2 subdivision of the Proteobacteria (41). Currently 58 species and three subspecies are known, of which about 30 species are human pathogenic (18). *L. pneumophila* represents the predominant cause of legionellosis (24, 41, 66). The gram-negative coccobacilli are 0.3 - 0.9 µm wide and 2 – 20 µm long. While the bacteria show the coccobacilli form in clinical samples, filamentous forms are visible after growth on culture media (24). They are motile microorganisms with polar or lateral flagella (41). Furthermore, *Legionella*-like amoebal pathogens (LLAPs) exist, which do not grow on routine culture media, but can be isolated by co-cultivating with their protozoan host cells. Recently, three LLAP strains were named *Legionella* species (18, 24, 41).

Legionellae are found worldwide in freshwater environments like rivers, streams, ponds, thermal pools and other moist biotopes like soil, mud or the canopy of the rain forest and in man-made water systems (10, 18, 24, 41, 130, 146). In their natural habitats, they are rarely associated with legionellosis (10, 24, 130). Most cases of legionellosis can be traced to man-made water systems with water temperatures higher than the ambient temperature (24, 41). Due to the anthropogenic change of the environment from the mid of the 20th century, legionellosis emerged in the last half of the 20th century (41). Legionellae exist in environments with a pH range of 2.7 - 8.3 able to withstand exposure to pH 2.0 for a short period (146). The bacteria survive at temperatures of 0 – 68 °C (24) and multiply between 20 °C and 45 °C (24, 130). Growth is inhibited above 55 °C, and above 60 °C the bacteria die. Below 20 °C they rarely multiply (130). The bacteria cannot survive in dry environments (41).

The biochemical metabolic properties are characterized by the positive catalase reaction, the production of beta-lactamase, the absence of urease and nitrate reduction and the ability to

liquefy gelatine. Amino acids serve as a carbon source for chemoorganoheterotrophic growth in the obligate aerobic milieu (41).

Legionellae are fastidious bacteria and in their natural aquatic environment they are present in biofilms where growth is enabled by co-existing microorganisms providing nutrients and as intracellular parasites of biofilm-grazing protozoan host cells (24). The host cells include the genus *Acanthamoeba, Naegleria* and *Hartmanella*, two ciliates species of the genus *Tetrahymena* and one species of a slime mould (146). Virulence factors affect the ability to legionellae to grow in host cells (66). The survival of legionellae in the host cells depends on the ambient water temperature: at 22 °C the bacteria are digested and at 35 °C they replicate (146). Protozoa protect Legionella from biocides and thermal disinfection(146). *Legionella* can multiply in host cells and leave them after temporal pore-formation-mediated lysis or they can remain within an encysted amoeba, where they are able to survive adverse environmental conditions and airborne aerosols (146). During the intracellular phase, proteins are produced that facilitate the infection of new host cells (146). The infection of human macrophages is the consequence of the prior adaption to intracellular growth within protozoa (10).

1.1.1.2 Transmission

The deep inhalation of small legionellae containing particles of 5 µm in diameter moving over a distance up to 12 km can cause legionellosis. Special meteorological conditions with thermal inversion promote a vertical mixture of aerosols and the horizontal transport (31, 36, 164). Less common modes of transmission are microaspiration of contaminated water, direct contact with surgical wounds (18, 24, 66). Person-to-person transmission is exceptional (6). The infective dose of *Legionella* to cause illness is unknown (5, 114).

After inhalation of *Legionella* contaminated aerosols, the bacteria bind to receptors of the Dot/Icm type IV secretion system on the surface of macrophages in the alveolar mucosa of the lungs and enter in the host cell cytosol. They resist phagocytosis by inhibiting the fusion of phagosomes with lysosomes, establish a replicative *Legionella*-containing vacuole, multiply in the vacuole and are released in the extracellular space accompanied by apoptosis of the host cell leading to necrosis. The bacteria can infect other macrophages and invade large areas within the lungs (30, 63, 66).

The reservoirs, life cycle and mode of transmission of *Legionella* are shown in Figure 1.

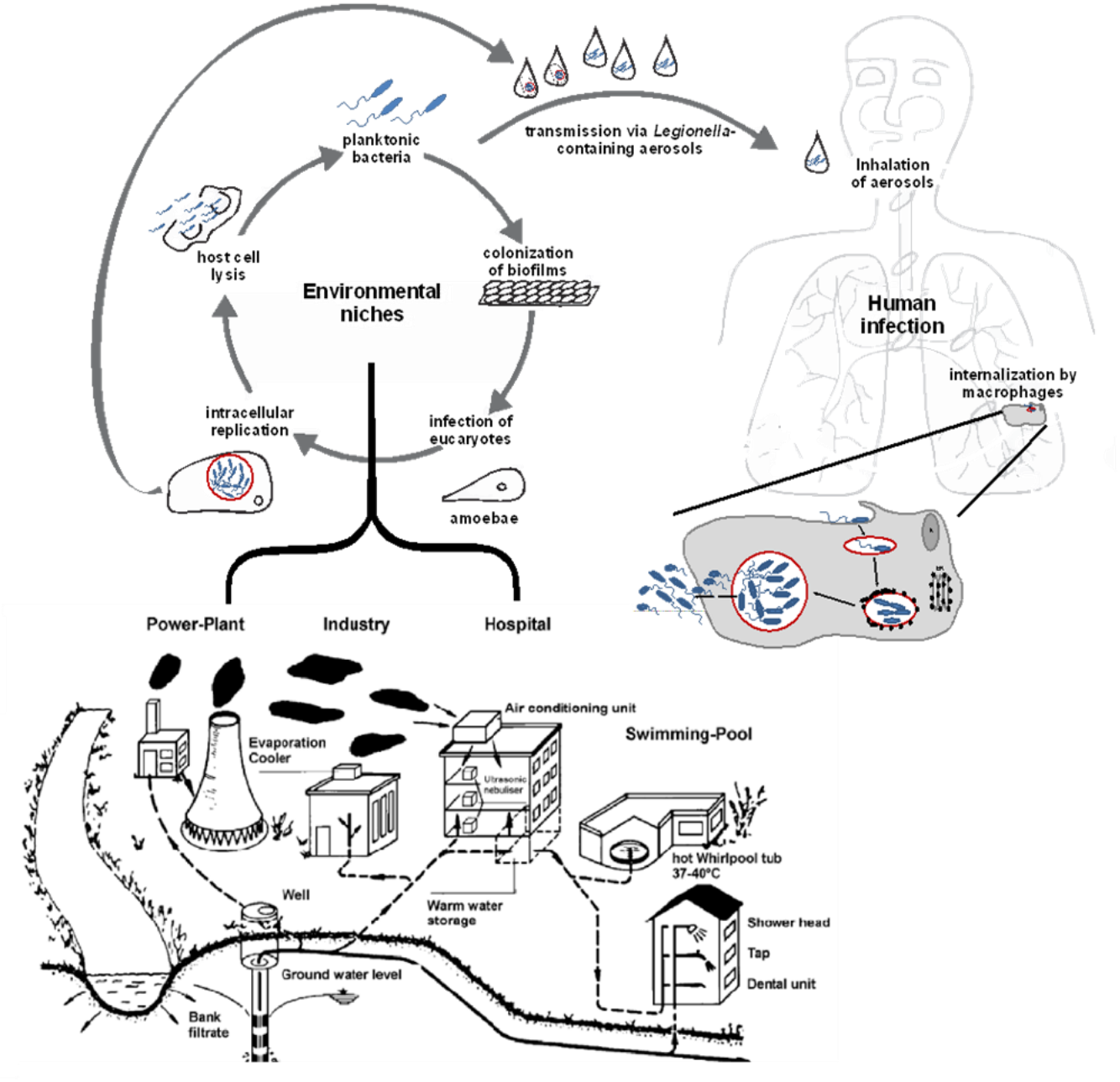


Figure 1: Life cycle, reservoir and mode of transmission of *Legionella*.
In their natural aquatic reservoir and in man-made water systems, *Legionella* exist in biofilms, where they are able to infect eukaryotic cells like amoebae. After replication, *Legionella* cells are released in the water. *Legionella*-containing aerosols are inhaled into the lungs and internalized by macrophages, where they avoid digestion but form a replicative vacuole. *Legionella* are released from the macrophages by necrosis ready to invade new macrophages of the alveolar mucosa (24, 30, 41, 63, 66, 130, 145). Figure modified and adapted from Hilbi et al., 2011, (63), Eisenreich and Heuner, 2016, (30), and Prof. Dr. Dr. M. Exner at Forum Städtehygiene, 1988.

1.1.1.3 Incidence, virulence and symptoms

The two distinct clinical entities caused by *Legionella* are the Legionnaires' disease (LD), a pneumonia with severe multisystem disease, and the flu-like, self-limiting Pontiac fever (24, 51, 66, 91, 103, 130).

The exact incidence of Legionnaires' disease worldwide is unknown, mainly because countries differ in awareness levels, diagnostic methods, and reporting (18). Legionnaires' disease accounts for 2 – 15 % of all cases of community acquired pneumonia (145). Approximately 70 % of all legionellosis are community acquired, 20 % are travel-associated and 10 % healthcare related (6, 130). The case-fatality rate amounts to 5 – 10 % (130). Common sources for community acquired, travel associated and nosocomial legionellosis are cooling towers, hot- and cold-water systems, spa pools, thermal pools and springs (66, 123).

Since all *Legionella* species are presumably able to reproduce intracellularly in host cells, it is likely that most species can cause human disease under the appropriate conditions. So far, about half of the known species were associated with human disease (41). The German competence network for community acquired pneumonia was able to show that the majority cause of infection of legionellosis is *L. pneumophila* predominantly serogroup 1. Round about 10 % of the infections are caused by other *Legionella* species (163). Also in the US, *L. pneumophila* has been identified as the causative agent of all legionellosis in about 90 % (41). Particularly the differently termed MAb3/1-, Dresden monoclonal type 3/1, Joly monoclonal type 2 (MAb2) or Pontiac subtype is responsible for 85 % of all cases of LD caused by *L. pneumophila* serogroup 1 (24). After *L. pneumophila* most infections are caused in immunosuppressed patients by *L. anisa*, *L. micdadei*, *L. bozemanii* and *L. dumoffii* (18, 19, 130). In Europe, 17 cases of combined *Legionella* species co-infections were observed between 2002 und 2012 (168). Cases of Legionnaires' disease occur worldwide. With a reported incidence of 1.7 cases per 100,000 inhabitants (2018), Germany is slightly below the current European average of 1.8 cases per 100,000 inhabitants (32, 131). Since not all pneumoniae are tested for legionellosis, is to assume that the disease is under-reported. The actual number of community acquired cases of legionellosis in Germany is estimated at 15,000 to 30,000 per year (163).

The incubation period of Legionnaires' disease amounts to 2 - 10 days and the illness lasts for weeks. In the initial phase of LD anorexia, malaise and lethargy are characteristic symptoms. Fever, dry cough (later with pus-forming or blood-streaked sputum), chest pain and gastrointestinal symptoms are typical symptoms as well. Almost half of the patients develop disorders related to the nervous system. The symptoms worsens during the first week, if untreated, and may lead to shock and respiratory, renal and multi-organ failure (66). The early therapy with

antibiotics (macro-azolides, tetracyclines, fluorchinolones and ketolides) promote a full recovery (24, 66, 130).

The Pontiac fever is called after Pontiac, Michigan, where an outbreak of a non-pneumonic febrile illness has occurred in July 1968 (51). The incubation period amounts to five hours to three days (most commonly 24 – 48 h) and the illness lasts for 2 – 5 days. Pontiac fever has a high attack rate up to 95 %. Deaths are not associated with Pontiac fever. It is an Influenza-like illness with the typical symptoms like asthenia, tiredness, high fever, chills, myalgia, headache, arthralgia, diarrhoea, nausea, dyspnoea and dry cough. A supportive treatment aims at relieving symptoms. Antibiotic therapy is not necessary (66). Due to its benignity and the unspecific symptoms, Pontiac fever is under-diagnosed and underreported (24). Furthermore, it is a matter of debate, whether Pontiac fever is caused by *Legionella* due to the too short incubation period to enable high *Legionella* multiplication in the body (24). Additionally, the absence of pneumonia, short duration of the milder illness and recovery without antibiotics are unlikely for a *Legionella* infection (24). These facts and the high frequency of the positive antigen urine test against *L. pneumophila* suggest to experts that Pontiac fever is probably due to exposure to a toxic mixture of lowdose live or dead *Legionella* bacteria incapable of causing pneumonia in combination with endotoxins of other live and dead microorganisms (24).

1.1.1.4 Risk factors

The exact risk factors leading to outbreaks or cases of LD are not completely understood, but the presence of virulent bacteria in an aquatic environment, multiplication and transmission of the bacteria via aerosol increase the risk of LD (24).

People with immunosuppression or chronic diseases of the heart or lungs, with diabetes mellitus, smokers and elderly people are particularly affected and at risk. Men develop the disease two to three times more frequently than women (18, 24, 41, 66, 130, 145).

The temperatures in the cooling system, the biofilm formation and the aerosolisation of respirable particles, especially under special meteorological conditions with thermal inversion, are factors increasing the risk for legionellosis sourced by cooling towers (36, 42).

An increase in legionellosis occurs regularly during the summer and autumn months (130). Travelling during the holiday season and the associated risks of infection (e.g. staying in hotels, swimming in whirlpools, etc.) as well as stagnation of water in the pipes of the home during a possible holiday might be related to this seasonal phenomenon (130). Furthermore, the higher temperatures in summer and autumn might promote the growth of legionellae in cold water or cooling towers and thus increase the risk of infection (130).

1.1.2 Biofilm formation in cooling towers

Evaporative cooling systems that produce possibly *Legionella*-containing aerosols are of infectious epidemiological importance, since any industrial systems generating water aerosols should be regarded as potential sources of contamination for Legionnaires' disease (13, 112). Evaporative cooling systems dissipate waste heat from numerous processes of industrial process engineering plants, building ventilation systems, power plants, computer centres, refrigeration plants etc. into the ambient air (159). There are different types of evaporation cooling plants. Evaporative cooling systems with wet cooling towers with open or closed cooling circuits and cooling plants with adiabatic pre-cooling (159). In wet cooling towers with open circuit, the circulating cooling water is in direct contact with the air (159). In closed systems, the circuit cooling water is cooled in the heat exchangers and is not in direct contact with the ambient air (159). However, the water used to absorb the heat from the heat exchangers is in direct contact with the ambient air and is therefore comparable to an open system (159). The adiabatic pre-cooling of the air supplied to the heat exchanger by humidification is another cooling principle (159). There are also cooling towers with a dry cooling system in which the cooling medium in the heat exchanger is cooled exclusively by air and no aerosols are produced (159). There are many combinations of wet and dry cooling systems for efficient operation (159). Figure 2 shows a schematic illustration of a wet cooling tower with open circuit.

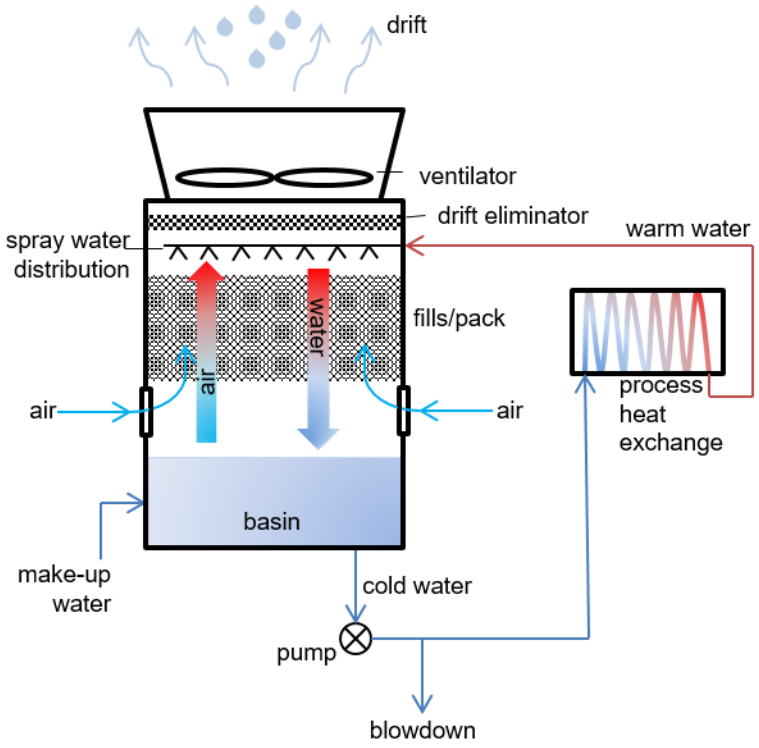


Figure 2: Schematic illustration of a wet cooling tower with open circuit.

Warm water from the heat exchange process is sprayed through the sprinkler nozzles and drops down along the fills or packs increasing the surface area into the basin. The fan draws ambient air into the cooling tower through openings in the lower part of the cooling tower. The warm water is cooled against the countercurrent of the air. The drift eliminator reduces the droplets in the exhausting warm air saturated with water vapour. Due to evaporation the concentration of dissolved salts in the water increases and precipitation solids may occur. A blowdown of the water is made at regular intervals to remove precipitations. The volume of the water lost through evaporation, windage and blowdown is replaced by the make-up water (11, 159).

Evaporative cooling systems provide ideal conditions for biofilm formation:

- **ambient air**: since evaporative cooling systems act as air washers the intake of organic matter and other debris from the air can accumulate in the cooling water increasing the nutrient availability in the cooling tower (42, 159)
- **water temperature**: usual temperatures in an evaporative cooling tower system range from 22 °C to 35 °C allowing growth of mesophilic microorganisms like *Legionella* and their protozoan hosts (42)
- **construction**: the construction material and the design regarding the surface and stagnation areas influence the biofilm formation on all wet or moist surfaces on heat exchangers, on the fills, in the basin and in the pipe network of the evaporative cooling system (11, 42)
- **source water quality**: microorganisms and substrates can be introduced into the cooling system via the make-up water (94). While make-up water originating from the municipal water supply has low loads of microorganisms and nutrients, the loads increase for make-up water originating from surface water like rivers, lakes, streams or reservoirs (42).
- **water treatment**: in the dynamic environment of a cooling tower, temperature, pH, conductivity, total dissolved salts, precipitations, suspended matter and biological mass can change in a short period of time and may influence the biofilm formation (11, 42) and many water treatment chemicals, like antiscalants and corrosion inhibitors, provide nutrients accelerating microbial growth (94).

In general biofilms form everywhere (96) and bacteria prefer to live in biofilms (100). Biofilms are defined as matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces including microbial aggregates and floccules (17). Biofilms consist of micro-colonies of sessile cells, embedded in dense matrix of extracellular polymeric substances (EPS) interspersed with open water channels (16). The EPS determines both the structure and cohesive strength of biofilms (144). The EPS represents 85 % of the biofilm mass (126). Bacteria

in micro-colonies have developed into organized communities with functional heterogeneity (15). There are many microniches in the biofilm. Within 100 µm, the environment can change from aerobic at the edge of a microcolony to completely anaerobic inside the microcolony. This results in a remarkable structural heterogeneity of species that benefit from their metabolic properties in a symbiotic consortia (16). The biofilm facilitates nutrient and gaseous exchange, and protects microorganisms not only from biocides but also from periodic increases in temperature and attempts at physical removal, especially in areas where surfaces are scaled or corroded (146). The metaphorical symbolisation that a biofilm represents a "city of microbes" (166) and the EPS represents the "houses of the biofilm cells" (45) vividly emphasises the complexity of the microbial relationships in the biofilm. The EPS plays an important role in the biomechanical properties of the biofilm and consists of many more substances than polysaccharides (formerly EPS stood for extracellular polysaccharide substance). A more appropriate name for EPS is extracellular polymeric substance, because EPS consists in addition to polysaccharides of biopolymers such as proteins, glycoproteins, glycolipids and extracellular DNA. In environmental biofilms the polysaccharides even represent only a small fraction (45).

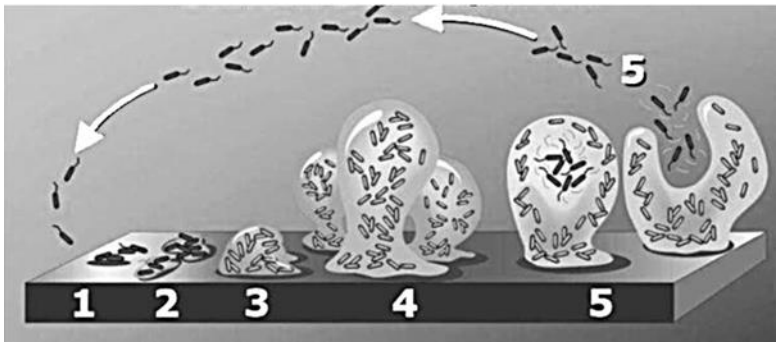


Figure 3: Biofilm formation.
1: initial attachment of cells to the surface and formation of micro-colonies; 2: production of EPS; 3: growth and early development; 4: maturation; 5: dispersion of single cells or parts of the biofilm. Adapted from Stoodley et al., 2002 (144).

Figure 3 shows the stages of the biofilm formation. The first step is the initial attachment and reversible adhesion of cells to the surface via type IV pili and flagella (144, 166). The rougher the surface, the higher is the extent of microbial colonisation, as shear forces are decreased (26). To stabilize irreversibly on the surface the extracellular polymeric substances are produced. The biofilm formation occurs by the redistribution of attached cells by surface motility, by the binary division of attached cells and by the recruitment of cells from the bulk fluid (166). As environmental conditions change within the young biofilm and the surface becomes covered by bacteria, secondary (planktonic) colonizers are able to attach to the primary colonizers and the biofilm begins to develop into a multi-species community (128). During the biofilm maturation process that lasts up to ten days the basic micro-colony/water channel architecture is developed (144). Liquid flow occurs in these water channels, allowing diffusion of nutrients,

metabolites, oxygen, and even antimicrobial agents (26). Many cells alter their physiological processes according to their microniches. The biofilm structure is determined by the production of the EPS, the growth rate, twitching motility, cell signalling and the physical and chemical conditions. Depending on the flow conditions, the biofilms appear differently. Isotropic towers or mushroom shaped micro-colonies are formed under no or low laminar flow conditions. Under higher flow with increased shear, filamentous biofilms are formed elongated in the downstream direction. Furthermore, the physical consistence of biofilms depends on the shear. Biofilms grown at higher shear are more, rigid, smoother and denser than those grown at low shear. During the late maturation process micro-colonies detach from the biofilm and leave empty spaces and individual cells leave the biofilm and become planktonic closing the biofilm developmental cycle (144).

As mentioned above, in biofilms microorganisms are protected against biocides. A variety of mechanisms of resistance are known. The interaction between biocides and biofilm cells and EPS results in the reduced penetration of the biocide into the biofilm and is called reaction-diffusion interaction mechanism (137). Under stress conditions (nutrient limitation, environmental stress, exposure to sublethal amounts of biocide), the gene expression and regulation changes leading to stress-resistant phenotypes. These phenotypes grow slowly and they are more resistant to the presence of biocides, pH changes, cold and heat shock (137). Depending on the location of the cells in the biofilm, different conditions prevail and different phenotypes of the same species may exist (137). Quorum sensing regulates the gene expression depending on changes in population density and acts as communication tool (137). Quorum sensing bacteria produce and release chemical signal molecules (autoinducers) that increase in concentration as a function of cell density (137). The detection of a minimal threshold stimulatory concentration of an autoinducer leads to an alteration in gene expression both within and between bacterial species influencing symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation (106). Quorum sensing may influence the biofilm resistance by affecting the biofilm formation and/or the regulation of gene expression of products involved in resistance (137). The occurrence of “persister cells” which represent special phenotypic variants in a population, selected in mature biofilms under certain conditions (they are no mutants as they appear in 10 to 10,000 higher rates than mutants), may influence the biofilm resistance (137).

Biofilms affect the interaction between metal surfaces and the environment. Microorganisms attached to the metal surface mediate biofouling and corrosion. Microbial adhesion processes lead to an important modification of the metal/solution interface and may drastically change the electrochemical features (160, 161). The thickness of the biofilm formed onto the heat transfer surface may reach 1 mm or more resulting in an insulating layer drastically reducing

the effectiveness of the cooling system and causing costs for additional energy requirement (11).

In general microbial infections are caused in 65 - 80 % by cells from biofilms (126). Due to their aerosol transmission, the presence of legionellae in cooling tower biofilms is of particular interest. Legionnaires‘ disease community outbreaks are often associated with cooling towers (2, 12, 24, 31, 37, 38, 41, 47, 49, 50, 54, 58, 59, 66, 68, 83, 85, 87, 101, 112, 113, 120, 124, 133–135, 138, 164, 167, 169). They often occur in autumn and small towers (< 300 kW) are often implicated in outbreaks (9). So far little is known about the microbiological relationships in biofilms, especially the role of legionellae (95). To cause an outbreak colonization, survival and proliferation of *L. pneumophila* in a cooling tower is essential (117). *Legionella* are secondary colonizers of biofilms (116). The bacteria localize in three distinct spatial arrangements in a biofilm: either contained within or directly associated with protozoa, or dispersed in loosely associated clusters or in tightly packed aggregations of cells forming dense colonial clusters. The formation of discreet clusters of tightly packed *Legionella* suggests that colony formation is influenced by specific environmental conditions allowing for limited extracellular replication (147). Legionellae are protected in the biofilm against environmental impacts and additionally they are able to survive and replicate in biofilm-grazing amoebae (21). Biofilm-associated legionellae are more resistant than the same bacterial species in the water phase of the system (146). The planktonic population of legionellae in cooling towers is seeded directly from sediments and biofilms located in the warmer areas of the system pipework and these sites may be the major areas of *Legionella* multiplication within the system (9).

Neglect or insufficient maintenance are the major risk factors for the proliferation of biofilms and in particular of *Legionella*. The restarted operation of systems without appropriate biocide treatment were in the past often the reason for LD outbreaks (42, 164). Registration systems for evaporative cooling systems were demanded early on (31, 36). With the publication of VDI 2047-2, the “VDI Cooling Tower Code of Practice” in 2015 (159), and the introduction of the German 42nd Ordinance Implementing the Federal Immission Control Act (42nd BImSchV) in 2018 (1) as well as the land registry office for evaporative cooling systems in 2019 (KaVKA), the fundamentals were created to prevent legionellosis outbreaks. Furthermore, in case of an outbreak faster actions to identify the source are enabled. The 42nd BImSchV regulates the laboratory testing of *Legionella* spp. and of heterotrophic plate counts (HPC) (1). The VDI 2047-2:2015 recommends the additional testing for *Pseudomonas aeruginosa* (159).

Heterotrophic microorganisms include bacteria, yeasts and moulds and require an organic carbon source for their proliferation. Many culture-based tests exist to recover the range of organisms able to grow under the given nutrient and incubation conditions (172). A sudden or

continuous increase in the number of colonies indicates problems and must be clarified by further investigations to find the cause (55). The heterotrophic plate count technique is useful to assess the efficacy of antimicrobial treatments of cooling tower water (42).

P. aeruginosa is known for being involved in the initial process of biofilm formation by adhering on surfaces type IV pili-mediated and its ability to produce extracellular polymeric substances (7, 111, 137, 144). Thus, the detection of the bacterium in cooling tower water is of particular interest and recommended by VDI 2047-2:2015 (159). The Gram-negative, facultative anaerobic growing, rod-shaped bacterium has a unipolar flagellum and is ubiquitous in aquatic environments (39). In biofilm formation, the production of the EPS is of particular importance as it protects bacteria from antibiotics and disinfectants (15, 39). *P. aeruginosa* is an opportunistic pathogen, naturally resistant against a lot of antibiotics and able to proliferate in man-made water systems, particularly in outlet fittings and sinks of wash basins (39). The bacterium mainly affects immunocompromised patients and is a particular problem in hospitals representing 8 – 11 % of nosocomial infections in Europe and the United States (7). Typical clinical pictures are chronic pneumonia in patients with cystic fibrosis, infections in skin burns and otitis media in swimmers. Nosocomial infections usually occur in intensive care patients as urinary tract or lung infections (7, 39). In cooling towers, the presence of *P. aeruginosa* indicates a massive surface colonisation due to insufficient disinfection and maintenance possibly leading to process-relevant impairments (159).

These two parameters, *P. aeruginosa* and HPC, are used as monitoring parameters in cooling towers. Increased concentrations are not consulted as a health hazard, but give reason to check the system with regard to its operating mode (159).

The removal of biofilms from industrial water systems is problematic and the emphasis has been placed upon control rather than removal as the more realistic option (147). Chemical and physical methods exist to reduce the biofilms in cooling tower systems. While chemical treatment is often noxious, physical treatment is not appropriate for each systems (11, 159). Physical treatment includes the mechanical cleaning and the use of filters or ultrasonic. Rare physical treatment methods comprise the circulation of balls, brushes or sponge rubbers through the pipe system, or flexible plastic tubes that oscillate to shake off deposits. Mechanical cleaning is performable when the cooling tower or cell(s) of the cooling tower is not in operation and therefore depends on the operation mode of the cooling tower. Even if a different physical treatment is applicable, the chemical treatment is essential. The biocide treatment must be adapted to the conditions of the cooling system able to reduce biofilm formation (11).

A biocide is a chemical agent that inactivates pathogenic and non-pathogenic organisms (137). Biocides are applied in various locations to prevent, inhibit or eliminate microbial growth

(137). In the control of biofouling, the biocide treatment aims usually to reduce microorganisms on a surface in order to restore or maintain the correct function of a technical system (137). The biocide efficacy is determined by its biocidal effect and the ability to remove biofilm (137). Many oxidizing and non-oxidizing inorganic and organic biocides are known. They are usually used in commercial formulations. A number of biocides are able to remove biofilms from surfaces (137).

Oxidizing biocides:

In cooling water systems frequently used oxidizing biocides include chlorine, bromine, stabilized bromine, combinations of bromine and chlorine, chlorine dioxide, peroxy compounds such as hydrogen peroxide and peracetic acid, and ozone (42). Oxidizing antimicrobials are often effective when fed continuously using metering systems with small pumps, and many towers are successfully treated with continuous dosing with chlorine or bromine (42). Shot-dosing of oxidants, which can also be very effective in microbial control, is an alternative to unvarying application of oxidizing antimicrobials (42).

Non-oxidizing biocides:

Non-oxidizing biocides should be shot dosed to be most effective (42). The continuous presence of non-oxidizing biocide residuals in the system will promote the selection of resistant microorganisms and loss of microbial control (42). Non-oxidizing biocides are usually dosed at higher concentrations (15 – 50 ppm) than oxidizing biocides, and may require longer contact times at these concentrations (4 – 10 h) (42).

In general, biocides have a broad spectrum activity and multiple targets contrary to antibiotics, which tend to have specific cellular targets (137). It is not easy to elucidate the exact mechanism of action of a biocide (137). More than one cell constituent is often affected, and consequently to distinguish the primary effect from secondary effects is impeded (137). Biocide targets comprise the cell wall, cytoplasmic membrane and ribosomes of vegetative cells, the coat and cortex of bacterial spores, the envelopes and capsids of viruses, structural proteins, enzymes, nucleic acids and polysaccharides (137). The biocide attack results in the disrupture of membranes with leakage of intracellular components, the destruction of some cellular functions like replication, transcription, protein synthesis, and metabolism (137).

1.2 German regulations for evaporative cooling systems

The German 42nd Ordinance Implementing the Federal Immission Control Act (42nd BImSchV) (1) rules in accordance to the German Cooling Tower Code of Practice VDI 2047-2:2015 (159) the routinely laboratory testing of *Legionella* spp. and of heterotrophic plate counts in the water of cooling towers and scrubbers in regular intervals. The German Federal Environment Agency (**U**mwelt**b**undes**a**mt, UBA) has published a recommendation in which the exact laboratory testing procedure is explained. The recommendation was first released in 2017 and a revised version was adopted in 2020 (155, 156). The focus of interest is the protection of the public health. The best way to limit *Legionella* concentrations is good maintenance and regular checking of *Legionella* concentrations (5, 31, 34, 35, 42, 164). Therefore, a method that is easy to handle and provides reliable results is of great importance. The *Legionella* detection of industrial waters with the UBA-recommended ISO 11731:2017 procedures is often hampered by the high contamination of interfering bacteria.

The *Legionella* concentration determined in a water sample is compared with the test and action values of the 42nd BImSchV (see Table 1). Depending on which value has been exceeded, the operator has to repeat laboratory investigations, clarify the cause of exceedance, establish the correct operational mode, introduce weekly internal company inspections and to take protective measures to prevent hazards. The test and action values of the 42nd BImSchV are based on the relative risk assessment of Kenepp and Miller (107). Their relative risk assessment is derived from concentrations of *L. pneumophila* detected in cooling towers, which were associated with LD (107).

Table 1: Test and action values of the German 42nd Ordinance Implementing the Federal Immission Control Act (42nd BImSchV).

System type	Test value 1	Test value 2	Action value
	Legionella spp. concentration [cfu/100 mL]		
Cooling towers (evaporative cooling systems)	100	1,000	10,000
Scrubbers	100	1,000	10,000
Cooling towers, cooling capacity > 200 MW (often natural draft cooling towers)	500	5,000	50,000

For HPC, reference values for both incubation temperatures are formed from the mean values of six successive results. If the reference values are exceeded by a factor of 100 or more, the operator must clarify the cause and implement measures to reduce microbial contamination. The value of 10,000 cfu/mL is allowed to be used as well (1).

1.3 Microbiological detection methods in cooling water samples

There are numerous methods to detect microorganisms in water samples like culture, fluorescence in-situ hybridization, quantitative polymerase chain reaction, direct fluorescent antibody test, flow-cytometry and immunomagnetic separation but the cultivation of microorganisms on nutrient media remains the gold standard (41).

The 42nd BImSchV regulates the laboratory testing of *Legionella* spp. and of heterotrophic plate counts (1). The VDI 2047-2 recommends the additional testing for *P. aeruginosa* (159). The respective ISO methods are permitted.

The ISO 11731:2017 (80) decision matrix for the detection of *Legionella* spp. in water matrices includes numerous and complex steps for cooling water samples due to the expected high amount of microbial contamination. The German Federal Environment Agency (UBA) has issued a recommendation with a reduced number of procedures accredited laboratories have to apply for the detection of *Legionella* spp. in cooling water samples. A wide range of possible concentrations must be covered by using different sample volumes, as *Legionella* generally occurs over a wide concentration range (0 - > 100,000 cfu/100 mL) in cooling tower water samples. *Legionella* colonies are mostly greyish in colour and have different morphologies. Since legionellae grow slowly, they are often overgrown by colonies of accompanying interfering microorganisms. These both facts complicate the examination of grown plates. Presumptive colonies must be confirmed and the time from sample processing to the final result can take up to 14 days.

The DIN EN ISO 6222:1999 (73) for the detection of heterotrophic plate counts was primarily developed for the detection of cultivable microorganisms in water matrices with low microbial contamination. If the microbial concentration is expected to be high, dilution series should be carried out (73). Considering the facts that microorganisms in solution do not form ideal solutions but are diffusely distributed and that the ISO makes few methological specifications, the method allows a wide scope of interpretation. Because only a very small volume is used to determine the heterotrophic plate counts, it is questionable how meaningful the investigation is for the detection in cooling water samples.

The DIN EN ISO 16266:2008 (77) is a membrane filtration method for the detection of *Pseudomonas aeruginosa* in water samples using a selective nutrient medium. Membrane filtration techniques are suitable for water matrices that contain few particles or colloidal substances (81). Thus, the counting of *P. aeruginosa* cfu is often hampered.

The company IDEXX Laboratories, Inc. (“IDEXX”) provides most probable number (mpn) methods for the specific detection of microorganisms in different water matrices. Two IDEXX

methods for the detection of *P. aeruginosa* and *E. coli* / coliform bacteria have already been ISO-standardised for potable water samples (78, 82). Mpn methods are approved in the quality control of water and food hygiene (39). In mpn procedures, a defined sample volume is incubated in several reaction vessels. From the relative frequency of positive reactions, the probable bacterial count in the sample is calculated (39). IDEXX mpn-tests are characterised by the fact that a large number of reaction chambers are filled in the so-called trays containing numerous reaction chambers in one single step. Specific substrates are used as the basis for the tests, which are metabolised by the target organism and lead to a measurable signal. Prior to the comparative study, carried out at the IHPH (142), Pseudalert was not yet approved for cooling water testing. By now, after internal laboratory validation, Pseudalert has also been licensed for other water matrices such as cooling water (82).

1.3.1 Detection of *Legionella* spp. and *Legionella pneumophila*

In Germany, the official detection method for *Legionella* spp. is the UBA recommended combination of procedures of the ISO 11731:2017 culture plating method. The IDEXX method Legiolert™/Quanti-Tray® provides an appropriate alternative method for the detection of *L. pneumophila* in water samples. In two multi-laboratory studies the performance of Legiolert was compared to ISO procedures of potable water samples (136, 141). Data from both multi-laboratory studies according to ISO 17994 (79) showed that Legiolert compared to the former ISO 11731-2 method (74) yielded on average higher counts of *L. pneumophila* (136, 141). The comparison with the former ISO 11731 (72) was inconclusive due to the number of samples needed to be tested. Likewise, comparisons of the MPN method for 100 mL to the highest result of either ISO 11731 or ISO 11731-2 according to the former UBA recommendation (2012) (154) yielded no conclusive difference, regardless of whether *Legionella* spp. (including *L. pneumophila* and non-*pneumophila* species) or only *L. pneumophila* were included in the evaluation (141). Because of the advantages of the IDEXX method compared to the ISO culture plating methods, it is desirable that Legiolert also offers good performance for cooling water samples. The advantages of Legiolert are providing results at seven days, rapid sample preparation and analysis, and objective interpretation of test results. In another study, the performance of Legiolert with the method of the Center for Disease Control and Prevention (CDC) for the detection of *L. pneumophila* from non-potable samples, mainly from cooling towers, was compared (127). The results demonstrated no significant difference between Legiolert and the CDC method, but Legiolert showed a significant increase in sensitivity for water samples containing higher *L. pneumophila* concentrations. Cooling tower waters often contain high amounts of interfering bacteria influencing *Legionella* culture plating methods. Legiolert was resistant to this interference and produced a very low rate of false-positive results (127). Based

on these results, a comparison of Legiolert and the UBA recommended procedures of the ISO method was carried out as part of this dissertation.

1.3.1.1 UBA recommended ISO 11731 procedures

The ISO 11731:2017 procedures recommended by the German Federal Environment Agency (155, 156) display a combination of membrane filtration and direct plating steps for samples with possibly high *Legionella* counts and possibly high contaminations with interfering bacteria. To cover a broad range from low (< 100 cfu/100 mL) to very high bacterial counts (> 10,000 cfu/100 mL) three different volumes (0.1 mL, 1 mL and 20 mL) are used. Inhibition of interfering bacteria is achieved by acid and heat pretreatment steps and the usage of highly selective GVPC agar (buffered charcoal yeast extract agar containing glycine, vancomycin, polymyxin B, and cycloheximide). Eight plates from seven volume and pretreatment steps are incubated at 36 ± 0.5 °C and examined for *Legionella* growth after three to five, seven and ten days. Typical colonies grown on the GVPC agar plates are then sub-cultured prior to confirmation of identity by serology. The serology testing was done in this study for each colony type, but UBA recommends serology testing only for *Legionella* spp. concentrations above 10,000 cfu/100 mL.

1.3.1.2 Legiolert

A novel alternative method has been developed based on the most probable number (MPN) determination of *L. pneumophila* in water. Legiolert™ is presented as powdered reagent in blister pack format for testing water samples and utilizes a selective and diagnostic substrate formulation to detect *L. pneumophila*. Quanti-Tray®/Legiolert™ is incubated at 37 ± 0.5 °C for seven days in a humid environment. *L. pneumophila* produce any combination of brown pigment and turbidity and represent a confirmed detection result. Enumeration is achieved by reference to a table for the most probable number (MPN) for the number of positive wells in the Quanti-Tray/Legiolert. The non-potable protocol of Legiolert is designed for 1 mL sample volume. The first test value of the German Ordinance is 100 cfu *Legionella sp*./100 mL. Testing only 1 mL with Legiolert would cause an exceedance of the first test value with each detection of a positive well and this would lead to actions of the cooling tower/scrubber operator. To cover the range between 10 and 100 mpn additionally 10 mL were tested with an adapted protocol from IDEXX.

1.3.2 Detection of heterotrophic plate counts

The detection of heterotrophic plate counts (HPC) has its origin in the drinking water analytic and goes back to Robert Koch (172). At that time, epidemics triggered by contaminated drinking water played a major role. Koch and his contemporaries were able to prove empirically

that colony counts below 100 cfu/mL did not cause epidemics in the effluent of slow sand filters (172). This empirical value is still used as a limit value for drinking water. At that time, the parameter had public health relevance. Today the parameter plays a traditional role in monitoring the drinking water treatment and the pipeline network (172).

Only a small proportion of the metabolically active microorganisms present in a water sample are detected under the given conditions. The actual organisms recovered in HPC testing can also vary widely between locations, seasons and consecutive samples at a single location (172). There are different HPC methods resulting in different counts and detected populations (90, 172). The 42nd BImSchV (1) recommends the performance of the ISO method (73).

The use of the two incubation temperatures of 36 °C and 20 to 22 °C, depending on the method, detect potentially hygienically relevant species at 36 °C, while typical environmental bacteria grow at the low temperature. For cooling tower water the test is primarily performed to detect a significant increase of the microbial concentration in the system (1, 73). Common microbial concentrations in cooling water samples are often in the range of 10^2 to 10^7 (28, 173). *Legionella* concentrations and HPC do not correlate and hence HPC are not applied as indicator parameter for *Legionella* (27).

1.3.3 Detection of *Pseudomonas aeruginosa*

The German official detection method for *P. aeruginosa* displays the DIN EN ISO 16266:2008 (77). This method is a membrane filtration procedure for samples with expected low bacterial contaminants. Cooling tower water often contains high numbers of interfering bacteria, which can impede the counting of *P. aeruginosa* colonies resulting in an underestimation. Thus, ISO 16266 does not offer an ideal enumeration method for *P. aeruginosa* from this matrix.

With ISO 16266-2:2018, the IDEXX mpn method Pseudalert has already been approved as the standard method for the detection of *P. aeruginosa* in potable, non-carbonated bottled, ground-, swimming pool and spa pool waters. The method is employable for other water matrices by undertaking appropriate validation performance (82). The suitability of Pseudalert for cooling water samples was carried out at IHPH: Pseudalert represented a significant improvement in the enumeration of *P. aeruginosa* from cooling tower water and related samples for 10 and 100 mL sample volume. The advantages of Pseudalert became apparent in a better performance, a more than 10-fold higher upper quantification limit when using Quanti-Tray/2000 and a shorter incubation time with no requirement for further confirmation, resulting in a faster reporting of results (142).

1.3.3.1 DIN EN ISO 16266:2008

A selected sample volume is filtered through a 0.45 µm pore size membrane filter. The filter is placed on a selective cetrimide-containing agar (Pseudomonas CN agar) and incubated at 36 ± 2 °C for 40 ± 4 h. Blue-green pyocyanin-producing colonies are *P. aeruginosa* and do not need further confirmation. Presumptive colonies that are fluorescent but non-pyocyanin producing or are reddish or brown in colour require further testing to demonstrate the production of ammonium from acetamide using Nessler's reagent.

1.3.3.2 Pseudalert

The powdered Pseudalert reagent utilizes a fluorogenic aminopeptidase substrate which is hydrolysed by *P. aeruginosa* leading to blue fluorescence under UV irradiation (365 nm). Pseudalert/Quanti-Tray is incubated at 38 ± 0.5 °C for 24 – 28 h. Enumeration is achieved by reference to a table for the MPN for the number of positive wells in the Quanti-Tray. The method has a reported sensitivity of 94 %, selectivity of 100 %, false-positive rate of less than 1.0 % and a false-negative rate of 6.5 % (82).

1.4 The four investigated cooling towers of this study

To observe the biofilm formation in cooling towers of different construction and biofilm treatment, the company Evonik has generously provided four cooling towers for study purposes. As agreed, the location of the cooling towers and details of the operation mode are handled anonymously. The observation period lasted ten months. The biofilm formation in the four cooling towers was investigated over a period of ten months between February and November 2019. It was examined how the biofilm on stainless steel and polyethylene plates developed in the turbulent flow in the basin. The biofilm formation was observed successive each four weeks and over the entire investigation period from the date of installing the plates. To install the plates in the turbulent flow of the cooling tower basin, a plates holding unit was designed. The construction of the plates holding unit is described in section 2.3. The unique opportunity arose to observe the biofilm development in Cooling Tower 1 under deactivated biocide treatment.

Retrospective data of these four cooling towers were analyzed to check whether the microbiological parameters form a function of the process parameters. In general, the typically recorded process parameters pH level, temperature, total hardness and turbidity are possibly associated with Legionellae growth (4). The pH, conductivity and DOC values of the four cooling towers showed to less fluctuations (data not shown) to enable a prediction of microbial concentrations. The analyses of retrospective *Legionella* (seven years) and HPC (two/three years) in combination with redox potential and water temperature data were performed. The

prediction of microbial levels on the basis of process parameter data could enable an improved risk management, with even more rapid implementation of measures to prevent increased *Legionella* concentrations and thus Legionellosis outbreaks.

The cooling towers of this study were treated exclusively with oxidative biocides. The supplementary make-up water is obtained from a canal. After treatment, it is thickened several times in the cooling systems in the form of partial decarbonisation and is weakly alkaline.

The control of the biocide dosage was generally carried out depending on the redox potential. The redox potential describes the oxidizing or reducing properties, i.e. the amount of electrons donated or accepted, in a system. It is measured in millivolts (mV) and generally by an inert metal electrode (e.g. platinum) in conjunction with a saturated calomel electrode or an Ag/AgCl electrode as reference electrode. These electrodes have defined redox potentials in relation to the standard hydrogen electrode, which by definition has a potential of zero. Systems which give off electrons to the inert metal electrode have a negative redox potential, those which accept electrons have a positive redox potential. The higher the redox potential, the more oxidative the environment. Due to microbial metabolism there are generally changes in the redox potential in a habitat and redox potential gradients depending on the location (140). Additional peracetic acid shock dosages were carried out in the case of dense algae growth or high *Legionella* spp. contamination.

Table 2 contains information on the construction, like the main building material used, the cooling water basin area and volume and the cooling water capacity as well as the biocide used.

Table 2: Fact sheet of the four investigated cooling towers.
[a] The systems consist of two spatially separated cooling towers. The information refers to the total cooling system and is unknown for investigated cooling tower exclusively.

CT	Material	Cooling units	Year of construction	Biocide	Biocide concentration [ppm]	Basin area of CT [m^2]	Basin volume of CT [m^3]	Capacity [m^3]	Max capacity [m^3]	Circular-flow rate [m^3/h]
1	Concrete, plastic	5	1959	Chlorine dioxide	0.3	1.374	2.115	2.200	5.000	3.500
2	Plastic	4	2018	Ozone	-	2.470	5.900	22.000 (total) [a]	24.000 (total) [a]	25.500 (total) [a]
3	Wood, plastic, asbestos	1	1955	Chlorine	-	609	760	3.800 (total) [a]	8.000 (total) [a]	7.600 (total) [a]
4	Plastic, steel	4	1977	Chlorine, sodium bromide	-	1.110	1.700	2.800	5.600	5.600

1.4.1 Cooling Tower 1

The Cooling Tower 1 has a concrete/plastic construction and was built in 1959. It is an in-line arranged five-cell cooling tower treated with chlorine dioxide. The five cells cover an area of $11 * 11\,m^2$ and a basin volume of each $423\,m^3$.

Figure 4: Cooling Tower 1.

Cooling Tower 1 has the smallest capacity and circulation volume of the four investigated cooling towers. The in-line arrangement is shown in the upper picture of Figure 4. The inner concrete construction and the sprayed water (below the not visible fills) are apparent in the middle picture of Figure 4. The algae growth is noticeable on the concrete bars. According to the five units of Cooling Tower 1 five chlorine dioxide pumps are available. The chlorine dioxide dosage pumps are shown in the lower picture of Figure 4.

Chlorine dioxide has been used in drinking water disinfection treatment since 1944 (139). Until 2012, it was hardly used in cooling towers due to the high effort involved in its production. With the development of a new process for the production of chlorine dioxide, it was increasingly used for the treatment of cooling towers (14). Chlorine dioxide is produced in situ from hydrochloric acid and sodium chlorite for use in cooling towers (56). Since it is very volatile, it is well distributed in cooling circuits. Chlorine dioxide is a highly water-soluble, toxic gas. It does not dissociate, but is present in water as a free radical. Therefore, its effect is pH independent. Although it is less reactive than chlorine, it is more effective than chlorine in an alkaline environment and no organically bound halogens (AOX) or other organic disinfection by-products are formed (14, 29). Chlorine dioxide has the ability to dissolve biofilms, to prevent their formation and to eliminate algae (14, 44, 139).
During the observation period, the unique opportunity arose to observe the biofilm development in Cooling Tower 1 under deactivated biocide treatment.

1.4.2 Cooling Tower 2

The largest of the investigated four cooling towers is Cooling Tower 2 with a maximum capacity of 24,000 m^3 and a circular flow rate of 25,500 m^3/h. In total, the cooling system comprises two cooling towers, each consisting of four cells. As Cooling Tower 2 was built in 2018, the retrospective analyses were performed for the microbial concentrations of the other system. The (maximum) capacity and the circular flow rate refer to the entire cooling system. Only the investigated cooling tower is a plastic construction from 2018, with each of the four cells measuring 14 * 14 m^2. The biocide treatment of Cooling Tower 2 is ozone. The pictures of Figure 5 show parts of Cooling Tower 2. During the entire observation period, filamentous algae grew on the inner construction. These filamentous algae are visible in the lower image of Figure 5.

Figure 5: Cooling Tower 2.

Ozone is a very effective, oxidative biocide. It must be produced on site. At pH values above 8, ozone breaks down into highly effective O_2 radicals, which react quickly with numerous inorganic and organic substances. This is accompanied by a decreasing disinfection effect (29). It could be shown that dissolved ozone at concentrations between 0.1 and 0.3 ppm was able to eliminate planktonic cells within a contact time of 10 and 30 min (25, 161). The advantages of ozone are the final decomposition products of O_2 and H_2O without any residuals persisting in the water under normal conditions (25). The formation of by-products like iodate and bromate has been observed (44). The O_2 radicals attack the unsaturated fatty acids of the cell membrane. In an in-vitro study ozone was able to inactivate *L. pneumophila* more rapidly than chlorine (25). Depending on the composition of the EPS, ozone can better or worse penetrate biofilms and act biocidal (137).

1.4.3 Cooling Tower 3

Constructed in the year 1955, Cooling Tower 3 is the oldest of the four investigated cooling towers. The outer shape reminds of an oversized coffee grinder as shown in the left image of Figure 6. The entire cooling system comprises two cooling towers. While Cooling Tower 3 consists of one cell, the other cooling tower consists of four cells, which together have a basin volume about twice as large as Cooling Tower 3. The round base area of Cooling Tower 3 could not be provided by the operator and was estimated via Google Maps (52) at 25 m in diameter. The known basin volume and the calculated radius accordingly estimate the basin area. The materials used are mainly wood, plastic and asbestos. On the lower picture of Figure 6 an algae layer is visible on the wall. The pictures were taken in February. In warmer months, a very thick carpet of algae was recorded on the entire wall surface.

The biocide treatment of Cooling Tower 3 is performed with chlorine. The disinfection of drinking water with chlorine was introduced in the early 1900s and has successfully reduced the incidence of waterborne diseases (48). The use of chlorine in drinking water treatment played an important role in the preservation and promotion of public health (48). Chlorine is a commonly used chemical for the control of biofouling (137). The term “chlorine” includes a mixture of Cl_2, OCl^-, HOCl and other active compounds whose efficacy depends on pH and temperature. (137). The disinfecting effect of chlorine is essentially based on its property as an oxidizing agent. The active agent is hypochlorous acid (HOCl), which predominates between pH 4 and 7. Hypochlorous acid (HOCl) is in equilibrium with the hypochlorite ion (OCl^-) depending on the pH value. Between pH 4 and 7, chlorine predominately exists as hypochlorous acid (HOCl) in equilibrium with the hypochlorite ion (OCl^-), which predominates above pH 9 (14, 137). The hypochlorous acid biocidal effect is caused by the denaturation of proteins. Some bacteria own the heat protein Hsp33, which provides a protective mechanism against hypochlorous acid. (171). The hypochlorite ion disrupts and detaches mature biofilms (137). While chlorine inactivates planktonic cells well, biofilm microorganisms persist in the presence of high chlorine concentrations. This is probably caused by chlorine consumption and neutralization due to the interaction of chlorine and the with cells and EPS in biofilms (137). The use of chlorine leads to the formation of by-products such as trihalomethanes, chloramines and other organic halogen compounds (AOX). The advantages of using chlorine or hypochlorite are its effectiveness through oxidation and its high toxicity to microorganisms. The disadvantages are side reactions with in-/organic compounds in the water resulting in chlorine consumption, the possible corrosion and the formation of environmentally harmful AOX, which can accumulate (29, 137). In the alkaline environment cooling towers are frequently operated, chlorine is insufficiently effective (14).

Figure 6: Cooling Tower 3.

1.4.4 Cooling Tower 4

In 1977, Cooling Tower 4 was mainly built with plastic and steel. The cooling tower consists of four cells arranged in pairs next to each other. Each cell has a base area of $9.5 * 12.8\,m^2$. The cooling water basins are accessible via ladders. As shown in the right picture of Figure 7, the inner bars are hardly covered with algae. The individual cells are similar in structure to those of Cooling Tower 1.

Figure 7: Cooling Tower 4.

Cooling Tower 4 is treated with a combination of chlorine and sodium bromide.

In the alkaline environment cooling towers are frequently operated, chlorine is insufficiently effective (14). To improve the biocidal effectiveness, chlorine can be used in combination with sodium bromide (14). The formation of hypobromic acid improves the biocidal effect(14). The chlorine/bromide combination has a strongly limited effectiveness against microorganisms in biofilms and algae (14). The use of peracetic acid can compensate for these disadvantages (14). In addition to disinfecting properties, it also has deposit-dissolving properties (14).

1.5 Aim of this dissertation

The aim of this dissertation is the improved cooling tower maintenance enabled by additional easier to handle microbial detection methods and by the development of prediction tools as well as the better understanding of biofilm formation in well maintained cooling towers.

Comparative studies of IDEXX mpn methods with the standardised ISO methods have been performed in order to have additional detection methods, which allow a better or at least an equally good detection of *Legionella pneumophila* and *Pseudomonas aeruginosa* in cooling water samples.

The prediction tools were developed using retrospective microbial (*Legionella* spp., heterotrophic plate counts) and process parameter data (redox potential, water temperature). One tool based on correlation analyses, in order to alert the cooling tower operator as early as possible to potential microbial changes traceable by process parameter data in the cooling tower. Additionally, an easy risk factor calculation using retrospective microbiological data was created.

Investigations on biofilm formation were performed in order to better understand microbial development in cooling towers of different biocide treatment and construction materials, especially with regard to the regularly monitored and important microorganisms (*Legionella* spp,, heterotrophic plate counts, *P. aeruginosa*) in four well-managed cooling towers. To evaluate the influence of the construction material and biocide on the biofilm formation microbial growth on stainless steel and polyethylene plates and planktonic microorganisms were analysed in four closely located cooling towers sourced by the same make-up water during a ten-month observation period.

2 Material and Methods

2.1 List of devices

Table 3: List of devices.

Device	Usage or note	Producer
100 mL Glass cylinder	Volumetric measurements	Schott AG, Mainz, Germany
250 mL sampling bottles	Collection of water samples, preparation of biofilm suspensions, containing 20 ppm sodium thiosulphate	LP Italiana spa, Milano, Italy
Black gridded cellulose mixed ester filter	Detection of *Legionella* spp.	Merck Millipore, Merck KGaA, Darmstadt, Germany
Filtration pump	Filtration of water samples	Vacuubrand GmbH & Co
Filtration system and filtration funnels	Filtration of water samples	Sartorius AG, Göttingen, Germany
Fridge 1 (sterile material)	Cooling of sterile agar plates and solutions	Philipp Kirsch GmbH, Willstätt-Sand, Germany
Fridge 3 (unsterile material)	Cooling of water samples, Quanti-Trays, grown agar plates	Robert Bosch GmbHGerlingen, Germany
Hot-air sterilizer	Sterilization of reusable, clean consumables	Heraeus, Hanau, Germany
Incubator 1	Incubation of agar plates (22 ± 2 °C)	Memmert GmbH und Co.KG, Büchenbach, Germany
Incubator Leg2	Incubation of agar plates (36 ± 2 °C)	Heraeus, Hanau, Germany
Incubator Leg3	Incubation of agar plates (36 ± 2 °C)	Heraeus, Hanau, Germany
Loops, 1 µL (single use)	Confirmation of Quanti-Tray positive wells	Greiner bio-one, Solingen, Germany
Magnetic stirrer	Production of tenfold acid solution	Velp Scientifica, Usmate Velate MB, Italia
Microwave	Cooking agar	Domo, Linea 2000, Herentals, Belgium
Petri dishes, 9 cm	Pouring plates for HPC Collection of Biofilm mass	Greiner bio-one, Solingen, Germany
Pipettes	different volumes	Sartorius AG, Göttingen, Germany Eppendorf AG, Hamburg, Germany Brand GmbH und Co.KG, Wertheim, Germany
Plate turntable "petriturn-M"	Plating on agar plates	Schuett-biotec GmbH, Göttingen, Germany
Polycarbonate Filter	Concentration of microorganisms in a water sample; 50 mm, 0.2 µm	Whatman Nucleopore, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany
Quanti-Tray/2000	Cultivation of *P. aeruginosa* using the IDEXX most probable number method	IDEXX Laboratories, Westbrook, Maine, USA
Quanti-Tray/Legiolert	Cultivation of *L. pneumophila* using the IDEXX most probable number method	IDEXX Laboratories, Westbrook, Maine, USA
Scale	Weighing of chemicals for tenfold acid solution Weighing of biofilm mass	Sartorius AG, Göttingen, Germany
Sealer PLUS 2	Sealing of IDEXX Quanti-Trays	IDEXX Laboratories, Westbrook, Maine, USA

Device	Usage or note	Producer
Spatula	Plating on agar plates (Drigalski spatula) Scraping biofilm from surfaces (1 cm length of bevelled edge	Own production unknown
Sterile single-use pasteur pipettes	Moistening of dry smear surfaces	Greiner bio-one, Solingen, Germany
Sterile workbench	Plating of bacterial suspensions on agar	Kojair, Mänttä-Vilppula, Finland
Thermometer	Measurement of water and air temperatures	Hanna Instruments, Woonsocket, Rhode Island, USA
Ultra sonic water bath	Removal of microorganisms from polycarbonate filter	Bandelin electronic GmbH & Co.KG, Berlin, Germany
UV lamp	Identification of PA (Pseudalert), presumptive *Pseudomonas* species (ISO 16266), non-*pneumophila Legionella* (ISO 11731)	Spectroline, Westbury, New York, USA
Vortex	Mixing of sample and biofilm suspension aliquots	IKA-Works Inc. Scientific industries
Water bath	Heat treatment of bacterial suspensions for the detection of Legionella Cooling down and keeping warm molten agar	Memmert GmbH und Co.KG, Büchenbach, Germany
White gridded nitrocellulose filter	Detection of *P. aerugionosa*	Merck Millipore, Merck KGaA, Darmstadt, Germany

2.2 Chemicals, solutions and nutrient media

Table 4: List of chemicals, solutions and nutrient media.

Chemical / Solution / Nutrient medium	Usage	Producer
Acetamide solution	Production of ammonium from acetamide by *P. aeruginosa*	Sifin Diagnostics, Berlin, Germany
Aqua dest.	Fill up smaller test volumes to 100 mL in Legiolert and Pseudalert testing	Own production
BCYE agar	Cultivation of *Legionella* spp. Ref. No. PO5072	Oxoid, Wesel, Germany
BCYE+AB agar	Cultivation of *Legionella* spp. Ref. No. PO5325A	Oxoid, Wesel, Germany
Cetrimide agar	Cultivation of *P. aeruginosa*	Oxoid, Wesel, Germany
Columbia blood agar	Confirmation of *Legionella* spp. growth Ref. No. PB5008A	Oxoid, Wesel, Germany
GVPC agar	Cultivation of *Legionella* spp. Ref. No. PO5074A	Oxoid, Wesel, Germany
Hydrochloric acid solution 37 %	Production of tenfold acid solution	Merck KGaA, Darmstadt, Germany
Legiolert reagent	Nutrient mixture for the cultivation of *L. pneumophila* using the IDEXX most probable number method	IDEXX Laboratories, Westbrook, Maine, USA
***Legionella* Latex Test Kit**	Identification of *L. pneumophila* Serogroups 1, range of 2–14 and some non-*pneumophila* strains. Ref. No. DR0800M	Oxoid, Wesel, Germany
McFarland Standard	$BaSO_4$ solutions of different concentrations for the description of the tubidity and for the estimation of the density of bacterial soutions	bioMérieux, Inc., Durham, North Carolina, USA

Chemical / Solution / Nutrient medium	Usage	Producer
Nessler's reagent	Detection of ammonium production	Sifin Diagnostics, Berlin, Germany
Onefold Acid Solution	Inactivation of interfering bactering in *Legionella* detection	Oxoid, Wesel, Germany
Plate count agar	Cultivation ofheterotrophic plate counts (yeast extract agar)	Oxoid, Wesel, Germany
Potassium chloride	Production of tenfold acid solution, CAS-No. 7447-40-7, MQ300	Merck KGaA, Darmstadt, Germany
Potassium hydroxide	Production of tenfold acid solution, CAS-No. 1310-58-3, MQ300	Merck KGaA, Darmstadt, Germany
Pseudalert reagent	Nutrient mixture for the cultivation of *P. aeruginosa* using the IDEXX most probable number method	IDEXX Laboratories, Westbrook, Maine, USA
Sodium thioglycolate	Inactivation of oxidizing biocides in a water sample, CAS-No. 367-51-1, MQ100	Merck KGaA, Darmstadt, Germany k
Sterile potable water	Suspension solution for smears Diluent solution for ISO 11731 procedures Production of 10-fold acid solution Legiolert Pseudalert	Own production
Tenfold Acid Solution	Inactivation of interfering bactering in *Legionella* detection	Own production (recipe see below)

Tenfold acid solution:

Solution A: 0.2 M HCl solution:

- add 17.4 mL 37 % HCl-solution to 100 mL sterile *Aqua dest.*

Solution B: 0.2 M KCl solution:

- add 14.9 g KCl (powder) to 100 mL sterile *Aqua dest.*

Tenfold acid solution:

- 7.8 mL Solution A + 50 mL Solution B

The pH must not be adjusted to 2 - 2.2 with 1 M potassium hydroxide solution, otherwise the acidifying properties of the solution are significantly reduced.

2.3 Plates holding unit for the biofilm experiments

To observe the microbiological growth in the cooling towers, plates made of stainless steel and polyethylene in the dimensions 20*20 cm^2 were used. Holes were drilled in two opposite corners of each plate. Below one of the holes, a corner of 1 cm^2 was engraved. In the two free corners, squares of 5 and 10 cm edge length were engraved. To ensure that the plates were always exposed to the same conditions during their stay in the cooling tower, a “plates holding unit” was created. The plates holding unit is shown in Figure 8. The left side of the figure shows the original unit before installation in the cooling tower, the right side shows a schematic drawing. Each unit contained five pairs of plates. Two units were installed in each cooling tower.

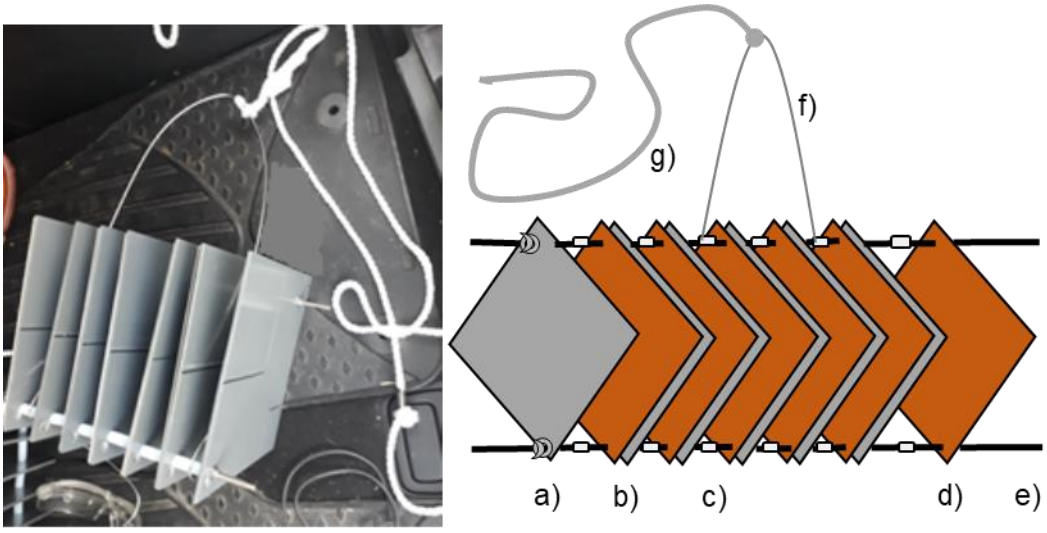


Figure 8: Plates-holding unit.
a) cover plate (stainess steel), b) pair of plates (stainless steel and polyethylene), c) plastic spacer, d) base plate (polyethylene), e) stainless steel bar, f) wire bow for fixing the g) rope.

Two stainless steel threaded rods formed the skeleton of the plate holding unit. A polyethylene plate served as the base plate (see Figure 8 d), which was fixed to the threaded rods (see Figure 8 e) with lock nuts and washers. Plastic spacers (see Figure 8 c) were used to separate the lowest pair of plates from the base plate and the other four paired plates (see Figure 8 b) from each other. The spacers prevent adjacent pairs of plates from touching each other. The top plate formed the stainless steel cover plate (see Figure 8 a). The stainless steel plate was fixed to the threaded rods with wing nuts. A waxed rope (see Figure 8 g) was knotted onto a wire bow (see Figure 8 f) that was attached to the threaded rod between the plate pairs. On each rope, the position was marked with knots and tape exactly one meter from the upper corner of the middle pair of plates. Thus, all plate holding units hung at a depth of 1 - 1.3 m. Only the units in Cooling Tower 3 hung at a depth of 50 - 80 cm due to the lack of basin depth. The description of the observation period and the procedure of the removal of plates and performing the swabs is given in section 2.4.2.

2.4 Sampling

The water samples of the retrospective analysis, of the comparative analysis of ISO and Legiolert and of the biofilm experiments were taken in accordance with the Quality Management Procedure Instructions of the Institute for Hygiene and Public Health (70, 71). The Procedure Instructions refer to the ISO standard on sampling for microbiological samples (75), the recommendations of the German Federal Environmental Agency on sampling and detection of *Legionella* in evaporation cooling systems, cooling towers and wet separators (155) and the VDI Code of Practice 2047-2:2015 (159).

2.4.1 Water samples of the comparative analysis of ISO and Legiolert

Between August and November 2018 industrial, naturally contaminated water samples (n = 99) were tested by the UBA recommended procedures of the ISO method (155) and Legiolert™/Quanti-Tray®. All samples were routine samples of the accredited Laboratory for Technical Hygiene at the Institute for Hygiene and Public Health at the University of Bonn. In total, 80 cooling tower samples, five scrubber samples and 14 samples from other industrial sites in total from 76 different sampling sites were tested to evaluate the comparability of the both methods. Samples were collected in 250 mL polyethylene or glass bottles containing 0.2 ppm sodium thiosulfate for the inactivation of oxidizing biocides. Isothiazolinone compounds were inactivated with 100 ppm sodium thioglycolate.

2.4.2 Water samples and swabs of the biofilm experiments

2.4.2.1 Water samples

Between February and November 2019, water samples were collected monthly from the four cooling towers for the biofilm experiments. Samples were collected in 250 mL polyethylene bottles containing 0.2 ppm sodium thiosulfate. The samples were taken from the cooling tower basins by diving the sample bottles in 30 cm water depth with the help of a sampling stick. The temperature was measured simultaneously.

2.4.2.2 Observation period

The biofilm formation in the four cooling towers was observed over a period of ten months between February and November 2019. On the one hand, it was examined how the biofilm develops every four weeks over the entire investigation period. For this purpose, a pair of plates was removed each month or each four weeks and replaced by a clean, disinfected pair. Results of the four-week short interval observation are described as "SI" for short interval. On the other hand, the observation of biofilm formation over the entire investigation period was carried out by monthly removal of one pair of plates, which has been persisting in the cooling

tower since its installation in February 2019. A spacer plate replaced the empty space in the unit. Results of the observation of the biofilm formation over the entire period are described as "LI" for long interval.

Until July, plates pairs were taken from the left-side hanging unit in the cooling tower basin. From August on, plates were taken from the second right-side located unit. The chronology of plate pair removal was clearly defined by the fact that plates were first removed from the unit hanging on the left side in the cooling tower and the upper and lower sides of the units were marked. Thus, the risk of confusion which paired plates had when to be removed was excluded.

2.4.2.3 Removal of plates

In order to remove the stainless steel and polyethylene paired plates from the plates holding units, the plates holding units were slowly pulled out of the water in order to avoid detachment of the biofilm. Without touching the inner plates, the unit was placed on the bottom plate on a clean surface. After the wing nuts had been removed quickly, the "cover plate" and any spacer plates were set aside. The two paired plates to be swabbed (one pair after four weeks in the cooling tower and one that had been in the cooling tower since the begin of the study) were carefully removed from the plates holding unit. By only touching the edges of the plates, it was prevented that the smear areas were touched.

2.4.2.4 Biofilm swabs

In the laboratory, the bottles for suspending the biofilm swabs were prepared by pouring 100 mL sterile tap water into sterile 250 mL polyethylene bottles containing 0.2 mg/mL sodium thiosulfate. The bottles were stored in refrigerated transport boxes until and after the swabs were taken.

After the removal of the plates as described above, the plates were placed with the marked side up on a clean surface next to the prepared bottles in which the biofilm smears were suspended. The 25 cm^2 areas were each swabbed with a stainless steel spatula of 1 cm edge length. The typical cotton swabs were not used, because the biofilms partly formed very tough coatings, which could not be removed in preliminary tests with a cotton swab. The stainless steel spatula was cleaned and disinfected with alcohol wipes before and after each plate.

To obtain as much biofilm material as possible, the 25 cm^2 area was scraped three times with a spatula. After the area has been completely scraped once or a lot of biofilm material stuck to the spatula, the spatula was dipped into the sterile water on the inside of the bottle. The biofilm was scraped off on the vessel wall and it was suspended in the water. Then the spatula was swivelled vigorously in the water and moved over the bottom of the bottle. Before the spatula

was pulled out of the bottle, it was well tapped at the edge of the bottle opening. This procedure was repeated until the area was scraped three times. The marked areas were scraped off as quickly as possible in order to prevent the plates from drying out. In some cases, very rapid drying of the plates was observed. In this case, either the spatula was wetted with sterile tap water from the corresponding target bottle before and partly during the scraping process or about 3 mL sterile tap water from the target bottle was dropped on the marked area on the plate using a sterile Pasteur pipette.

While the four swabs of the plates pairs were carried out, the plates holding units were reassembled in order to keep the time outside the cooling tower basin as short as possible. The reassembled plates holding unit was carefully returned to the cooling tower basin.

To cover a wide range of microbial concentration, dilution series of each biofilm suspension up to the level 10^{-4} were carried out in the laboratory by adding 0.5 mL of the biofilm suspension to 4.5 mL sterile *Aqua dest*.

2.5 Microbiological Methods

Water samples and biofilm suspensions were tested for *Legionella* spp. and *L. pneumophila*, heterotrophic plate counts (HPC) and *Pseudomonas aeruginosa*.

In the comparative study of Legiolert and the UBA recommended procedures of ISO 11731 (155), water samples were tested exclusively for *Legionella* spp. and *L. pneumophila*.

Results that were included in the retrospective analysis were obtained from routine water samples of the Laboratory for Technical Hygiene, which were tested exclusively for *Legionella* spp. and *L. pneumophila* until 2017. The screening for HPC was introduced in 2017.

For the biofilm experiments, water samples were screened for *Legionella* spp. and *L. pneumophila*, HPC and *Pseudomonas aeruginosa* using the corresponding ISO cultivation-based methods and the most probable number methods Legiolert and Pseudalert. The biofilm suspensions were tested with modified ISO methods for *Legionella* spp. and *L. pneumophila*, HPC and *P. aeruginosa*.

The performed microbiological tests of each study part with the corresponding methods are listed in Table 5.

Table 5: Microbiological methods performed in this work.
If presumptive *Legionella* colonies were detected with the (modified) ISO 11731 method, the colonies were checked with the latex agglutination test and could be assigned to *L. pneumophila* if agglutination occurred with the corresponding test sera.

Study part	*Legionella* spp.	*L. pneumophila*	Heterotrophic Plate Counts	*P. aeruginosa*
Comparative Analysis				
Standard method	ISO 11731 UBA rec. procedures	Identification via latex test	routinely partially recorded, data not shown	
Trial method	-	Legiolert	-	-
Retrospective Analysis	ISO 11731 "Bonn method" (Data 2012 - 2018)	Identification via latex test	Correlation with redox potential data: TrinkwV (2017 to 10.2018), ISO 6222 (11.2018 - 2019) Correlation with temperature data TrinkwV (2017 to 10.2018), ISO 6222 (11.2018 – 12.2018)	-
Biofilm experiments				
Four Cooling towers				
Water samples	ISO 11731 modified UBA rec. procedures	Identification via latex test	ISO 6222	ISO 16266
		Legiolert		Pseudalert
Biofilm suspensions	modified ISO 11731	Identification via latex test	ISO 6222	modified ISO 16266
No Biocide treatment				
Water samples	ISO 11731 "Bonn method"	Identification via latex test	ISO 6222	ISO 16266
		Legiolert		Pseudalert
Biofilm suspensions	modified ISO 11731	Identification via latex test	ISO 6222	modified ISO 16266

The volumes of the microbiological examinations carried out in the biofilm suspensions are listed in Table 6.

Table 6: Volumes of the microbiological tests of the biofilm suspensions.
MF: membrane filtration.

		Legionella* spp./ *L. pneumophila	**Heterotrophic Plate Counts**	***P. aeruginosa***
	Agar	GVPC + acid treatment	YEA	Cetrimide
Biofilm suspension	undiluted	20 mL MF 2 * 0.5 mL	1 mL	20 mL MF 2 * 0.5 mL
	10^{-2}	2 * 0.5 mL	1 mL	2 * 0.5 mL
	10^{-4}	-	1 mL	-

In Table 7 the detection and upper quantification limits and the degree of measurement uncertainty are shown for direct plating, membrane filtration and most probable number techniques according to ISO 8199 and UBA (81, 155). The detection and quantification limits of the final results obtained by the different methods depend on the sample volume and dilution level examined and the volume to which the final result was related.

Table 7: Quantification limits and measurement uncertainty of applied microbiological methods according to UBA and ISO 8199.
DL: detection limit; MU: measurement uncertainty; UQL: upper quantification limit; AMACC: as much as clearly countable, in this work, results were reported that exceeded the UQL according to UBA and ISO 8199 (81, 155) when colonies were clearly countable.
[1] different from ISO 8199.

Method	**DL**	**very high MU**	**high MU**	**low MU**	**UQL**
Agar plating cultivation methods					
Direct plating procedures	1 cfu	1 – 3 cfu	4 – 9 cfu	> 10 cfu	300 cfu AMACC[1]
Membrane filtration procedures	1 cfu	1 – 3 cfu	4 – 9 cfu	> 10 cfu	80 cfu (200 cfu) AMACC[1]
Inoculation procedures	1 cfu	1 – 3 cfu	4 – 9 cfu	> 10 cfu	300 cfu (without counting grid) 50,000 cfu[1] (with counting grid)
MPN cultivation methods					
Pseudalert (Quanti-Tray/2000)	1 positive well	1 – 3 positive wells	4 – 9 positive wells	> 10 positive wells	95 positive wells = 2,420 mpn/vol.
Legiolert (Quanti-Tray/Legiolert)	1 positive well	1 – 3 positive wells	4 – 9 positive wells	> 10 positive wells	95 positive wells =2,273 mpn/vol.

At this point, a note on the counting of target colonies on membrane filters should be made. According to ISO 8199 (81), the total number of grown colonies should not exceed 80 cfu and the number of target organism colonies should be higher than 10 cfu. The maximum number of detectable colonies were allowed to be higher or lower, depending on how the filter is grown.

In this studies, colony counts above the UQL were also used for quantification if they were clearly countable, especially for the detection of *P. aeruginosa*.

2.5.1 Detection of *Legionella* and *L. pneumophila*

The detection of *Legionella* spp. was carried out with different combined procedures of the ISO method (80). The detection of *L. pneumophila* was performed with Legiolert and serological latex testing of suspicious *Legionella* colonies cultivated by ISO method. Since all samples were processed before 2020 with UBA recommended ISO procedures, the UBA recommendation of 2017 was applied (155).

2.5.1.1 Detection of *Legionella* spp. by procedures of the ISO method

The combination of ISO procedures (80) for the detection of *Legionella* spp. from water samples in the different parts of this study is listed in Table 8.

Table 8: ISO 11731 procedures.
a) UBA recommended ISO procedures (155), b) modified combinations of ISO procedures, c) "Bonn method" (DAkkS accredited procedures).

Sample Volume [mL]	Membrane filter on plate or direct plating	Treatment	ISO 11731 procedure
a) UBA recommended ISO 11731:2017 procedures (comparative study of Legiolert and the ISO method)			
1 * 0.1 mL	Direct plating	None	1C
2 * 0.5 mL	Direct plating	Heat	2C
1 * 0.1 mL	Direct plating	Heat	2C
2 * 0.5 mL	Direct plating	Acid (10^{-1} dilution of the sample with onefold acid solution)	3C*
20 mL	Membrane filter on plate	Heat	6C*
20 mL	Membrane filter on plate	Acid	7C
b) modified combinations of the UBA recommended ISO 11731:2017 procedures (Biofilm experiments with the four cooling towers)			
1 * 0.1 mL	Direct plating	None	1C
2 * 0.5 mL	Direct plating	Heat	2C
1 * 0.1 mL	Direct plating	Heat	2C
2 * 0.5 mL	Direct plating	Acid (tenfold acid solution)	3C
1 * 0.1 mL	Direct plating	Acid (tenfold acid solution)	3C
20 mL	Membrane filter on plate	Acid	7C

c) "Bonn method" DAkkS accredited ISO 11731:2017 procedures; performed at IHPH (retrospective analysis and surveillance of the *Legionella* concentrations in one cooling tower under deactivated biocide treatment conditions)

100 mL membrane filtration, resuspension in 5 mL sterile potable water

2*0.5 mL	Direct plating	Heat	9C
2*0.5 mL	Direct plating	Acid (tenfold acid solution)	10C
2*0.5 mL 10^{-1} diluted resuspension	Direct plating	Acid (tenfold acid solution)	10C
2 *0.5 mL 10^{-2} diluted resuspension	Direct plating	Acid (tenfold acid solution)	10C

2.5.1.1.1 ISO procedures according to the UBA recommendation

For the evaluation of the comparability of the standard ISO method (80) and Legiolert (trial method) the ISO procedures according to the UBA recommendation 2017 (155) were performed. These procedures and the used sample volumes are described in Table 8 a.

Highly selective GVPC agar (buffered charcoal yeast extract agar containing glycine, vancomycin, polymyxin B, and cycloheximide) was used to reduce interfering bacteria.

To cover the test and action value ranges of the German 42nd Ordinance Implementing the Federal Immission Control Act (42nd BImSchV) the UBA recommends to test 0.1, 1 and 20 mL sample volume with different pretreatment steps to reduce the interfering bacteria. 0.1 mL of the origin sample was directly plated on GVPC without any treatment. 1 mL of the origin sample was treated with 9 mL acid solution (Oxoid, Wesel, Germany) and incubated for 5± 0.5 min. After incubation time 2*0.5 mL of the 10^{-1} diluted sample were plated on GVPC. Additionally, 20 mL of the sample were filtered through a black nitrocellulose filter with 0.45 µm pore size, incubated with 20 mL acid solution for 5 ± 0.5 min, washed with sterile potable water and placed on GVPC agar. Round about 25 mL of the origin sample were transferred in a sterile 50 mL tube and heat-treated in a water bath at 49 °C for 30 min, after the temperature was reached in a reference tube filled with 25 mL of sterile water. Of the heat-treated sample 0.1 mL and 2*0.5 mL were plated on GVPC agar and 20 mL were filtered through a black nitrocellulose filter with 0.45 µm pore size and the filter was transferred to GVPC agar. The eight GVPC agar plates were incubated at 36 ± 2 °C for ten days in a plastic bag to create a humid, carboxyphillic atmosphere, with examination after three to five, seven and ten days of incubation.

Confirmation of presumptive *Legionella* colonies

All plates were examined for growth of presumptive *Legionella* colonies and confirmation of each presumptive *Legionella* colony morphology type was done according to ISO by streaking material of presumptive colonies BCYE agar and tryptic soy agar with 5 % sheep blood (BA) (80). The ISO 11731 recommends, if one colony morphology type was present on the plate, the confirmation of three presumptive colonies should be done and if more than one colony morphology type was present on the plate, material of at least one colony of each type should be tested.

The confirmation procedure in this study differs slightly from the UBA recommendation (155) and the ISO 11731 (80) by implementing additional steps. In addition to the standard protocol outlined in the ISO, presumptive *Legionella* isolates of this study were screened for fluorescence under UV illumination to determine if isolates were *L. pneumophila* strains or non-*pneumophila* species of *Legionella*. *L. pneumophila* strains don't show any fluorescence (89). The isolates were additionally analysed by latex agglutination (*Legionella* Latex Test Kit, Oxoid) to identify *L. pneumophila* serogroup 1 strains, *L. pneumophila* strains from serogroups range 2-14 and non-*pneumophila* strains. Counts for *L. pneumophila* and for *Legionella* spp. were recorded separately.

Calculation of the final result

To calculate the final result according to the UBA recommendation (155), the condition with the highest *Legionella* colony count and the lowest concentration of interfering bacteria was chosen to reduce the influence of the measurement uncertainty.

Condition means the type of treatment in combination with the volume, e.g. 1 mL heat treatment including two GVPC agar plates or 0.1 mL non-treated sample plated on one agar plate. If all plates of the 0.1 and 1 mL heat-treatment were evauable, the weighted mean was calculated. In this study, the weighted mean was only chosen to calculate the final result, if the weighted mean of the colony count of both volumes was higher than the count of the 1 mL-heat-plates. This was done to reduce the measurement uncertainty of low counts of the smaller volume. Finally, the highest colony count was related to 100 mL according to the used volume to obtain the final result. The measurement uncertainty (low/high/very high) was recorded according to Table 7 and Table 8.

According to the UBA recommendation, a low measurement uncertainty is present if colony counts are higher than 10. A high measurement uncertainty is present when colony counts are between four and nine; a very high measurement uncertainty is present when colony counts are between one and three (155).

2.5.1.1.2 Biofilm experiments in four cooling towers

For the biofilm study, water samples and biofilm suspensions were examined for *Legionella* spp. and *L. pneumophila*.

2.5.1.1.2.1 Water samples

For the detection of *Legionella* in the 40 water samples from the four cooling towers the UBA recommendesd combinations of ISO 11731 procedures were modified (see Table 8 b). The membrane-filtration step of the heat treated sample was replaced by a direct plating step of 1 mL acid treated sample. Furthermore tenfold acid solution was used to treat the sample volume that was directly plated (the onefold solution was used for the treatment of the sample in the membrane-filtration step). Although the tenfold acid solution is not listed in the ISO 11731 (80), it has been used successfully for years in the Institute for Hygiene and Public Health at the University of Bonn in the accredited sector. The tenfold acid solution has the same ability to acidify the sample. However, the pH must never be adjusted during its production in the same way as for the onefold acid solution. Otherwise, the acidification capacity is significantly reduced. The advantage of the tenfold acid solution is that the volume of the sample is not increased tenfold reducing the measurement uncertainty.

The calculation and confirmation procedures of presumptive *Legionella* colonies for water samples of the biofilm study were carried out as described in 2.5.1.1.1.

2.5.1.1.2.2 Biofilm suspensions

After well shaking the biofilm suspension (production of the suspension is described in 2.4.2.4), 20 mL were filtered through a black gridded nitrocellulose filter and then covered with onefold acid solution for 5 min. Before the filter was transferred to GVPC agar after acid treatment, the filter was washed with 20 mL sterile tap water. In addition, 1,350 µL of each undiluted and 10^{-2} diluted biofilm suspension were incubated with tenfold acid solution (150 µL) for 5 min. After acid treatment, 2 * 550 µL each were plated out on GVPC. The agar plates were incubated at 36 ±2 °C for ten days in a plastic bag to create a humid, carboxyphillic atmosphere, with examination after seven and ten days of incubation.

The calculation and confirmation procedures of presumptive *Legionella* colonies of the biofilm suspensions were carried out as described in 2.5.1.1.1.

2.5.1.1.3 “Bonn method” performed for results of the retrospective analysis and surveillance in one cooling tower under deactivated biocide treatment conditions

Legionella results used for retrospective analysis and the *Legionella* results obtained during deactivated biocide treatment in one cooling tower were determined using the IHPH internal laboratory method (“Bonn method”). This method is approved by the Federal Environment Agency and accredited by DAkkS. The Bonn method is shown in Table 8 c.

After well shaking the water sample for at least 30 seconds, 100 mL were filtered through a polycarbonate filter. The filter was transferred to a sterile test tube with 5 mL sterile water (before the new ISO standard came into force in 2017 (80), distilled water was used, then sterile tap water). To improve the removal of microorganisms from the filter, the tube was placed in an ultrasonic water bath for 3 - 5 min at 80 W. After the ultrasonic treatment, the resuspension was mixed well. An aliquot of the resuspension was heat treated for 30 min at 49 °C. Serial dilutions of the resuspension were prepared for the acid treatment up to 10^{-2} with sterile water (*Aqua dest*. / tap water; see above), if necessary up to 10^{-3}. 100 µL of the tenfold acid solution were added to each 900 µL of the undiluted resuspension as well as to the dilutions, mixed and incubated for 5 min. 2 * 0.5 mL each of the heat and acid treated resuspension and the dilutions were plated out on pre-dried GVPC agar. Incubation was performed at 36 ± 2 °C in plastic bags to avoid dehydration of the agar plates and to create a carboxyphillic environment. After seven days the first examination of the plates was carried out.

Presumptive colonies (at least one colony of each morphology type or at least three colonies if only one morphology type was present) were checked by latex agglutination (*Legionella* Latex Test Kit) and fluorescence under UV illumination. If the latex test showed no or inconclusive reaction, the cysteine dependence was additionally checked with blood agar (48 h, 36 ± 2 °C). In the case of absence of growth on blood agar, corresponding colonies were assigned to *Legionella* spp. The equivalence of the latex agglutination test as a confirmation reaction was verified by corresponding parallel approaches in the laboratory for Technical Hygiene of the IHPH (data not shown). After the first examination after seven days, a second one was made additional three days later in order to detect possibly slow-growing *Legionella species*.

Calculation of the final result

Until March 2019, the dilution level chosen for the final result was the one with the highest result per 100 mL and at least ten grown *Legionella* colonies. If a high amount of accompanying flora was present on the plates with the most *Legionella* colonies, the next higher dilution level was used to calculate the final result. The accompanying flora may negatively influence the *Legionella* growth (155). The next higher dilution level plates were used only, if they provided the higher final result.

If less than ten colonies were present on the plates of each dilution step, the dilution step with the most *Legionella* colonies was used. If all plates had the same number of colonies, the dilution level that gave the highest final result was used. The results were related to 100 mL water sample. When filtering 100 mL sample, the number of colonies counted at each dilution stage was multiplied by five and the dilution factor to calculate the final result. If less than 100 mL were filtered due to the consistency of the sample, the determined colony count was related to 100 mL by the corresponding factor. The determined colony count was multiplied by factor 5, since resuspension of the filter was performed in 5 mL and 1 mL of the resuspension was examined.

From March 2018, the results obtained using the “Bonn method” were calculated as follows:

The raw data of all plates, any pre-dilutions, the filtration volume and the volume of the resuspension are entered into the raw data mask of the internal laboratory database "LegioData". The defined routine calculates the *Legionella* result from all specified values. First, the numerical values of the different dilution levels are related to 100 mL. For each sample pretreatment (acid / heat) the extrapolated value is noted, if this is also based on the highest number of actually counted colonies per dilution stage or if this number is higher than 20. In addition, a weighted average of the two lowest numerically evaluable dilutions of each pretreatment is recorded. The results of the different pretreatment steps carried out are compared and the result is used as the final result, followed by the highest number of colonies actually counted. This is not necessarily the highest result of all pretreatment steps. The extrapolation, which gives the highest result with the best analytical certainty is used as the final result (see UBA recommendation (155), Chapter E.4 and E.6). If only evaluable plates with three or less colonies are available, the weighted mean of this condition forms the final result.

If no *Legionella* colonies were present, the result was specified as < 5 / 50 / 500 cfu/ 100 mL depending on the clearly evaluable dilution level regarding the accompanying flora. If no dilution level was evaluable due to the presence of too high concentrations of accompanying microorganisms, the final result was determined as "not evaluable".

2.5.1.2 Detection of *L. pneumophila* by Legiolert

Legiolert is a commercially available most probable number testing system that consists of powdered reagent in blister pack format. Volumes of 10 and 1 mL were tested for each sample. Two vessels were filled with 100 mL and 90 mL sterile, deionizied water. The content of one blister pack of Legiolert™ reagent was added to each vessel. Non-potable samples require a pretreatment step. During pretreatment, the sample is acidified to inhibit interfering bacteria.

The 1 mL sample volume was pretreated according to the Legiolert protocol for non-potable samples as follows. After thoroughly agitation 2 mL of the sample were transferred to a sterile 50 mL tube and 2 mL of the onefold pretreatment solution were added, mixed and incubated for 60 ± 5 seconds. After the incubation time 2 mL of the pretreated sample were transferred to the vessel containing 100 mL dissolved Legiolert reagent.

The pretreatment of the 10 mL sample volume includes the following steps. After thoroughly agitation of the sample 10 mL were transferred in a sterile 50 mL tube (Greiner, Germany). 0.5 mL of the tenfold pretreatment solution were added to the aliquoted sample, mixed and incubated for 10 minutes ± 5 seconds). During the incubation time, the pretreated sample was mixed carefully. After the incubation time 0.5 mL Stop solution (30 % potassium hydroxide solution) was added to the pretreated sample, shortly agitated and immediately transferred to the vessel containing 90 mL dissolved Legiolert reagent.

After carefully agitation of the sample/reagent mixture for ≥ 60 seconds, it was poured in the Quanti-Tray/Legiolert® and immediately sealed in a Sealer model PLUS 2 using a Quanti-Tray/Legiolert rubber insert. Sealed Quanti-Tray/Legiolert samples were incubated paper side down at 37 ± 0.5 °C in a humidified environment using a closed plastic bag for seven days. Specific humidity level was not measured. Humidity is required to reduce evaporation of the wells.

Calculation of the final result

Quanti-Tray/Legiolert samples were examined after seven days for the presence of brown colour and/or turbidity. Enumeration was achieved by reference to a table for the most probable number (mpn) for the number of positive wells in the Quanti-Tray/Legiolert. The mpn values for the 1 and 10 mL sample volumes were related to 100 mL. The highest values (“max.”) and the “best” values were recorded for each sample. The “best” value of the two volumes per sample was calculated as follows according to the rules of ISO 8199:2018 and UBA recommendation (81, 155):

- the concentration of the Quanti-Tray/Legiolert that showed more than ten positive wells, even if the other tray with less than ten positive wells would lead to a higher concentration
- the concentration of the Quanti-Tray/Legiolert with the higher number of positive wells when both trays have less than ten positive wells
- the higher concentration of the Quanti-Tray/Legiolert when each of both trays has less than three positive wells

2.5.1.3 *Legionella* species and serotype testing via latex agglutination

Presumptive *Legionella* colonies grown on GVPC agar and 75 positive Legiolert/Quanti-Tray wells were confirmed by latex agglutination using the *Legionella* Latex Test Kit (Oxoid). The procedure was performed according to the manufacturer's instructions by suspending colony material with one drop each of buffer suspension and latex reagent on the test cards. In the positive case, a clear agglutination is visible without visual magnification after one minute. The quality controls were carried out in the Laboratory for Technical Hygiene according to the manufacturer's instructions and the requirements of the quality management of the Institute for Hygiene and Public Health. According to the manufacturer, the kit provides a screening procedure for *Legionella pneumophila* serogroups 1 and 2 - 14 as well as seven other *Legionella species* associated with human diseases.

The vials all contain a suspension of synthetic blue latex particles coated with specific rabbit antibodies against cell wall antigens of:

- Vial 1 (DR0801):
  - *L. pneumophila* serogroup 1
- Vial 2 (DR0802):
  - *L. pneumophila* serogroups 2 – 14
- Vial 3 (DR0803):
  - *L. longbeachae* 1 and 2
  - *L. bozemanii* 1 and 2
  - *L. dumoffii*
  - *L. gormanii*
  - *L. jordanis*
  - *L. micdadei*
  - *L. anisa*

Colonies showing a positive reaction with the antisera from vial 3 were recorded as "*Legionella* non-*pneumophila"* to distinguish them from other *Legionella species* that did not show any reaction in the latex test and which were recorded as "*Legionella sp.* or *spec*."

2.5.2 Detection of heterotrophic plate counts

With the publication of the VDI Cooling Tower Code of Practice in 2015 (159), the detection of heterotrophic plate counts (HPC) in cooling water samples became popular. In the first month after introduction of routinely HPC testing at the IHPH, 1 mL sample volume was analysed. Experience has shown that the use of 0.1 mL sample is more useful, since cooling water often contains very high concentrations of microorganisms and thus non-evaluable samples were reduced. Until November 1st 2018, the method was carried out according to the German Drinking Water Ordinance TrinkwV 2011, Annex 5 (148). After November 1st 2018, the method was

changed to the standard procedure ISO 6222 (73) on the basis of the decision of the Federal Working Group for Immission Control (Technical Module of the German 42nd Ordinance Implementing the Federal Immission Control Act) and the DAkkS. Only results of the retrospective analysis were influenced from the fact that different methods and volumes were carried out.

As part of the biofilm experiments, the detection of HPC was performed according to the ISO method (73). After shaking the sample bottle carefully, 0.1 mL of the water sample or 1 mL of the (diluted) biofilm suspension (as shown in Table 6) was pipetted into a petri dish (9 cm diameter) for each incubation temperature. To reduce material (plates and agar) and since the ISO does not give an exact instruction on how to count the colonies, Petri dishes with Wolffhügel counting grids were used instead of performing and plating more dilutions. The Wolffhügel counting grid is divided into 60 large squares, twelve of which are each divided into nine small squares and, thus, the upper quantification level is increased. The dilution levels and volumes used are shown in Table 6. Then the yeast extract agar, which had been liquefied by boiling and cooled down to about 45 °C, was added by pouring the warm, liquid agar not directly onto the sample but next to it. The sample was mixed with the liquid agar by tilting the petri dish in the shape of a figure of an eight. After the agar has solidified at room temperature, the agar plates were incubated hanging in an incubator at 22 ± 2 °C for 68 ± 4 h or at 36 ± 2 °C for 44 ± 4 h. The colonies were counted under bright illumination against a dark background using a microscope at tenfold magnification.

The method according to the German Drinking Water Ordinance (TrinkwV, Annex 5 I d, bb (148)) differs from the ISO method (73) in that

- DEV Agar is used as nutrient medium
- the lower incubation temperature is 20 ± 2 °C and
- the incubation time of 44 ± 4 h for both temperatures

Counting mode: up to 300 colonies per plate, the entire plate was counted (76, 81). If more colonies were visible and evenly distributed over the plate, parts (squares or small squares using the Wolffhügel counting grid) of the plate were counted and the number of colonies counted was multiplied by the corresponding factor to determine the final result as cfu/mL. Counting only one large square would mean that the colony number determined would have to be factorized with 60. If only one small square would be counted, the factor would be 9 * 60 = 540. In order to obtain a reliable result, at least three large or small squares were counted.

2.5.3 Detection of *P. aeruginosa*

The detection of *P. aeruginosa* was performed within the study assessing the biofilm formation in four different cooling towers. For water samples the ISO method (77) and the most probable

number method Pseudalert from IDEXX were applied. The biofilm suspensions were analysed by the modified ISO method.

The sample volume of the water samples was usually 10 mL. Depending on the result of the previous examination, 1 mL was examined additionally or exclusively. Especially for the ISO method 1 mL was used, because otherwise the upper quantification limit of 80 or 200 cfu would often have been exceeded.

2.5.3.1 ISO 16266 method

After well shaking the water sample bottle for at least 30 min, 10 mL sample volume was passed through a white nitrocellulose 0.45 µm pore size filter. The filter was transferred to Cetrimide agar and incubated at 36 ± 2 °C for 44 ± 4 h. If 1 mL was used as test volume, 1 mL sample volume was transferred into a 50 mL tube containing 20 mL sterile tap water. After well mixing, the sample was filtered and incubated as described above (77).

The biofilm suspensions were analysed by the modified ISO method. The 1 mL suspension volume was not filtered but plated onto two pre-dried Cetrimide Agar plates à 500 µL. The investigated sample volumes and dilution levels are summarized in Table 6. Incubation was performed as described above.

Blue-green pyocyanin producing colonies were counted as *P. aeruginosa*. Presumptive atypical *P. aeruginosa* colonies that were fluorescent but non.pyocyanin producing or were reddish or brown in colour were tested for the production of ammonium from acetamide with Nessler's reagent. Colonies producing ammonium were counted as *P. aeruginosa*.

2.5.3.2 Pseudalert

Sterile *Aqua dest*. was poured into a sterile 120 mL vessel. Using 10 mL sample volume 90 mL *Aqua dest*. were measured with a sterile glass cylinder. For a sample volume of 1 mL, *Aqua dest*. was filled up to the 100 mL mark of the 120 mL vessel. Pseudalert reagent was added to the *Aqua dest*. containing vessel. After the reagent was completely dissolved, the carefully shaken sample and Antifoam were added to the vessel. The sample/reagent-mixture was poured in a Quanti-Tray/2000 and sealed in a Sealer model PLUS 2 using a Quanti-Tray/2000 rubber insert. Sealed sample containing trays were incubated paper side down at 38 ± 0.5 °C for 24 – 28 h.

The trays were examined for the presence of blue fluorescence under UV light (365 nm). Enumeration was achieved by reference to a table for the most probable number (mpn) for the number of positive wells in the Quanti-Tray/2000.

Positive and negative controls were performed according to the Pseudalert instructions

2.5.4 Confirmation of isolate identity of positive Quanti-Tray wells

The specificity of Legiolert and Pseudalert was analysed by performing secondary confirmations. For confirmations of Legiolert positive wells, BCYE agar and tryptic soy agar with 5 % sheep blood (BA) were used. For confirmations of Pseudalert positive wells, Cetrimide agar was used. Within the comparative analysis of Legiolert and the ISO method, 783 positive wells were tested. During the biofilm experiments, 75 positive wells from numerous Quanti-Trays/2000 and Quanti-Trays/Legiolert were tested.

For each positive well, the sampling area on the paper/membrane side of the Quanti-Tray was identified, and both a razor and the sampling area were cleaned using a disposable alcohol wipe. The razor was used to cut a small opening in the paper/membrane above each well to be sampled and a loopful was transferred from each well to both the BCYE plate and the BA plate for Legiolert confirmations or to Cetrimide agar for Pseudalert confirmations. A 3-zone streak was performed for each aliquot on each plate, and the plates were incubated for 2 - 4 days at 36 ± 2 °C. The BCYE plates were incubated in a humidified atmoshphere by using plastic bags. Incubation time was variable based on recovery time for individual isolates to yield clear morphology and accurate confirmation.

2.5.5 Counting rules of the agar plated Biofilm suspension

The test volumes and dilution levels tested for *Legionella*, HPC and *P. aeruginosa* are shown in Table 6. The final result should be influenced by a minimized measurement uncertainty. Therefore a "cut-off" value of 10 cfu was used. The final result was calculated as described below. In the raw data files, the final result for samples without target organism detection was expressed according to the detection limit. In figures and calculations, missing detection was given as “zero” results, or as “one” in logarithmic charts.

The colony counts were related to the streaked area of 25 cm^2. For the detection of *Legionella* and *P. aeruginosa*, 20 mL biofilm suspension were passed through a membrane filter, 1 mL of the undiluted biofilm suspension and the 10^{-2} dilution were plated on agar. For the detection of HPC 1 mL undiluted biofilm suspension, the 10^{-2} and 10^{-4} dilutions were inoculated in liquid yeast extract agar.

To refer the final result to 100 mL suspenion or 25 cm^2 streaked area the colony count of the 20 mL membrane filtered biofilm suspension was multiplied by the factor 5. The colony count from the 1 mL plated biofilm suspension was multiplied by the factor 100. The colony count from 1 mL plated 10^{-2} diluted suspension was multiplied by the factor 10,000. The colony count from 1 mL plated 10^{-4} diluted suspension was multiplied by the factor 1,000,000.

To calculate the final result, the weighted arithmetic mean was calculated if counts ≥ 10 cfu were determined in at least two of the tested volumes. If only one tested volume showed more than nine colonies, this only colony count was used to calculate the final result.

If less than ten colonies were counted in one test volume, while the other test volumes showed no colonies, this count was used to calculate the final result. If less than ten colonies were given in several test volumes, the weighted arithmetic mean was used to calculate the final result.

2.6 Data analysis

2.6.1 Retrospective data analysis

The company operating the four cooling towers kindly provided the process parameter data. The retrospective analyses of the concentrations of *Legionella* compared to the process parameters (water temperature, redox potential) were carried out over the years 2012 to 2018. The retrospective analyses of the concentrations of heterotrophic plate counts (20 or 22 C / 36 °C) compared to the process parameter redox potential were carried out over the years 2017 to 2019. The retrospective analyses of the concentrations of HPC (20 or 22 C/ 36 °C) compared to the process parameter water temperature were carried out over the years 2017 and 2018. Over the entire period, 24 redox potential values per day were read out from the monitoring software and saved in an Excel file. For the years 2012 to 2016, four water temperature values were read out per day. For the years 2017 and 2018, 24 water temperature values were read out per day. The values were checked for plausibility. Non-plausible values (e.g. redox potentials higher than 1,500 mV or less than 20 mV, temperatures less than 4 °C) or zero measurements were removed from the data set. For each of the four cooling towers, an Excel file was created for each process parameter, containing all process parameter values and *Legionella* and HPC values for both incubation temperatures. The formulae in Excel have been created in such a way that the process parameter is observed over a selected period of time prior to each time of sampling; if relevant in combination with a threshold value of the process parameter that was regarded as useful. The process parameters were considered as independent variables, the microbiological values as dependent variables.

For the analysis of the microbiological parameters as a function of the process parameters,

- the unchanged microbiological results and
- the logarithmic values to base 10

were considered. The microbiological data were obtained from the laboratory database “LegioData”. In the original data, the final result for samples without target organism detection was

expressed according to the detection limit depending on which test volumes were clearly evaluable regarding the amounts of accompanying microorganisms. In figures and calculations of this study part, missing detection was given as “zero” results, or as “one” in logarithmic charts.

The redox potentials were considered over a certain period of time. To obtain a value against which the microbiological result could be plotted,

- the number of biocide dosages in the period before sampling,
- the number of biocide dosages in the period after sampling,
- the sum of biocide dosages before and after sampling,
- the ratio of the number of biocide doses (after/before sampling),
- the averaged redox potential before sampling and
- the period of measurements below the selected redox potential threshold before sampling

were calculated. The number of biocide dosages was determined by looking at the successive hourly-recorded redox potential values. If the subsequent value was higher than the previous value and above 400 mV, respectively 350 mV in Cooling Tower 2, it was assumed that biocide dosing had taken place. The biocide dosages were summed over the selected period.

The recorded water temperatures of the cooling water flow to the exchanger were also observed over a selected time. To obtain a value against which the microbiological result could be plotted,

- the mean temperature in the period before sampling,
- the minimum temperature in the period before sampling,
- the maximum temperature in the period before sampling,
- the period of measurements above the threshold temperature in the period before sampling,

were calculated.

The retrospective analyses were carried out in order to generate a tool that allows predictions of the *Legionella* concentration or the HPC due to changes in the water temperature or the redox potential. The prediction was generated by using a regression function. Although the data of the microbiological parameters were not normally distributed and an evaluation of the correlation according to Pearson is less suitable than the evaluation of the rank correlation according to Spearman, a regression analysis according to Pearson was performed (84). The Pearson correlation was applied since regression function was created, usually using the Pearson correlation coefficient to determine the quality of the correlation.

To evaluate a correlation of the data sets using a regression line, the mean values, standard deviations, variances, covariances, unexplained and explained variances were calculated. The

coefficient of determination was calculated from the ratio of the explained variance of the independent variable and the variance of the dependent variable and corresponds to the squared correlation coefficient according to Pearson (R^2). The Pearson`s correlation coefficient (r) induces no correlation when the value is close to zero, a weak correlation between ±0.1 and ±0.3, a moderate correlation between ±0.3 and ±0.5 and a high correlation when the value is between ±0.5 and ±1.0 (104). To assess whether the correlation of the data is statistically significant, the p-value was calculated after calculation of the t-value (84). The corresponding p-value for two-sided testing was obtained from the t-distribution using the "T.VERT.2S" function in Excel. Based on this calculation, Pearson correlation coefficients higher than |0.3| are considered statistically significant.

2.6.2 Evaluation of measurement uncertainty

Uncertainty of measurement evaluation and comparative enumeration analysis of the data was conducted according to the advice of ISO 8199 (81) and UBA recommendation (155).

2.6.3 Comparative data analysis

The comparative data analysis was performed within the framework of the method comparison for the detection of *Legionella* spp. and *L. pneumophila* in cooling water with Legiolert and the ISO reference method. Furthermore, it was used during the biofilm experiments to evaluate the suitability of Pseudalert and Legiolert for cooling water investigations by comparing them with their respective ISO reference methods. Additionally, the HPC from the Laboratory for Technical Hygiene were compared for the two incubation temperatures of 22 and 36 °C.

2.6.3.1 McNemar's test

The McNemar's exact binominal test (105) with the Yates correction for small data sets was used to evaluate the frequency of positive/negative paired data by the IDEXX trial methods and from the ISO method.

2.6.3.2 Comparative analysis according to ISO 17994

The mean relative difference approach of the ISO 17994 (79) served as the basis for the comparative analyses from the IDEXX trial methods Legiolert and Pseudalert and the UBA recommended procedures of ISO 11731 and the ISO 16266 (77, 80, 155). Furthermore, the mean relative difference approach was performed to the HPC from the Laboratory for Technical Hygiene determined with the ISO 6222 (73) for the two incubation temperatures of 22 and 36 °C.

The comparative paired count data of mpn results from the IDEXX methods and cfu results from the ISO methods were analysed according to the mean relative difference approach of

ISO 17994 (79) to determine whether the two methods could be considered of equivalent performance or not. Mpn counts from Quanti-Trays were converted to nearest whole integers. Data with counts that exceeded a method count limit by at least one method or had zero counts by both methods were excluded according to ISO 17994 (79). The mean relative difference was calculated by the mean of the relative differences (*x*) of each pair of counts using the equation

$$x = 100 \times (\ln(a) - ln(b)),$$

where ln(*a*) is the natural logarithm of the count by the trial method (IDEXX; HPC at 22 °C) and ln(*b*) is the natural logarithm of the count by the reference method (ISO; HPC at 36 °C). Data with a zero count by one method had “plus one” added to each pair of counts prior to log-transformation. Evaluation of equivalence is based on the mean relative difference and the expanded uncertainty (*W*) based on the standard deviation of the mean

$$W = \frac{2s}{\sqrt{n}},$$

from which the lower (X_L) and upper (X_U) limits of the “confidence interval” are calculated. The percentage value of the upper and lower limits was set at +20 % and –20 % as suggested by ISO 17994 (79). The methods are considered of equal performance if the lower limit of the confidence interval is between –20 % and zero, and the upper limit is between zero and +20 %.

According to ISO 17994 (79) the difference is significant if the total “confidence interval” is above or below zero. An “inconclusive” outcome is associated with the lower value of the “confidence interval” below zero but not below the set limit of –20 % and the upper value is higher than the set limit of +20 %. This is usually due to an insufficient number of samples being analysed and the influence of the multiplication of colony counts from small volumes to 100 mL.

2.6.3.3 Significance analyses

Before significance analyses were performed to determine whether data sets were different with a 5 % probability of error, tests for normal distribution were performed using the online tool (60). For paired samples, the Wilcoxon test (170) was conducted for non-normally distri-buted data and the paired t-test (150) was conducted for normally distributed data. For unpaired samples, the Mann-Whitney-U-test (99) was performed for non-normally distributed data. For unpaired samples whose data were normally distributed, the Lévène test (65) for equality of variance was carried out. For unequal variances, the Welch test (153) was performed, for equal variances the t-test (151) was applied.

Data analyses were performed using Excel 2010/2013 (Microsoft Inc., Redmond, Washington, USA) or online tools (60, 61, 65, 99, 150, 151, 153, 170).

3 Results

This chapter contains:

- the comparative analysis of Legiolert and the UBA recommended ISO 11731 procedures,
- the retrospective analyses of the process parameters temperature and redox potential and the *Legionella* concentrations and heterotrophic plate counts in four cooling towers,
- seasonal dynamics of *Legionella* and heterotrophic microorganisms cultivable under ISO 6222 growth conditons,
- the comparison of heterotrophic plate counts for the both incubation temperatures of 22 and 36 °C,
- the development of biofilms in four different cooling towers including a cooling tower specific risk factor calculation,
- the development of biofilm in a cooling tower under deactivated biocide treatment conditions.

3.1 Comparative analysis of Legiolert and the ISO method

The following section shows the results of the comparative analyses for the paired data of Legiolert and the UBA recommended ISO 11731 procedures (80, 155) including the McNemar's test (105) results and the mean relative difference approach of ISO 17994 (79).

In total, 99 samples were tested by both methods. Of the 73 Legiolert results calculated from the sample volume that provided the higher count ("max."), 17 originated from 10 mL sample volume and 56 from 1 mL volume. Of the 73 results calculated from the sample volume that was influenced from the lower measurement uncertainty ("best"), 38 originated from 10 mL sample volume and 35 from 1 mL volume. Regarding the UBA recommended ISO procedures, *L. pneumophila* was detected in 64 out of 99 samples. The ISO procedures from which the final results were obtained are shown in Figure 9. One final result derived from the 0.1 mL direct plating procedure without treatment, 35 from the heat treatment procedures and 28 from the acid treatment procedures. Regarding the heat treatment procedures, 19 final results were obtained from 1 mL direct plating procedure, six final results each were calculated from the membrane filtration and the weighted mean of the colony counts from the two direct plating volumes. Looking at the acid treatment procedures, 20 final results were calculated from the membrane filtration and eight from direct plating procedures. Total *Legionella* spp. (containing all detectable *Legionella* species including *L. pneumophila* without any specification) were detected in 69 samples. The distribution of the procedures that delivered the final result is almost congruent to that of the *L. pneumophila* detection. The number of final results obtained from

the 0.1 mL direct plating procedure without treatment, the heat-treated 0.1 mL direct plating and 20 mL membrane filtration with acid treatment procedures is equal. The final *Legionella* spp. results were calculated from the 1 mL direct plating procedure for 18 samples, from the combined 0.1 and 1 mL direct plating procedures for seven samples and from the direct plating with acid treatment procedure for twelve samples.

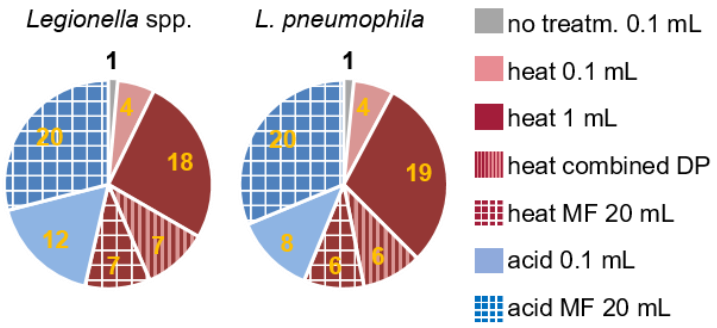


Figure 9: Distribution of the UBA recommended ISO procedures for the calculation of the final result.
DP: direct plating; MF: membrane filtration.

The methods' comparison was done for both the maximum ("max.") and the best Legiolert counts and the UBA-recommended calculation of the final result of the different ISO procedures for both *L. pneumophila* and *Legionella* spp. counts. As described in 2.5.1.2 the best Legiolert result represents the count of the two test volumes influenced by the lower measurement uncertainty. The data for all eight comparisons were analysed using the McNemar's test and the results were summarized in Table 9 to Table 12. The ISO and Legiolert counts are shown in the attachment.

The number of positive/negative paired data for max. and best Legiolert results were the same considering each the number of ISO *L. pneumophila* (see Table 9) and ISO *Legionella* spp. results (see Table 10). The McNemar's test outcome of the positive/negative paired data for the max. or best Legiolert results and the ISO *L. pneumophila* results shown in Table 9 indicated that the methods were different ($p = 0.039$) and that Legiolert was significantly more sensitive. The McNemar's test outcome of the positive/negative paired data for the max. or best Legiolert results and the ISO *Legionella* spp. results shown in Table 10 indicated that the methods are not different ($p = 0.409$).

To exclude results with a high measurement uncertainty from the McNemar's test, counts lower than three positive wells or cfu were counted as negative results (see Table 11 and Table 12). In Table 11, the McNemar's test outcomes of the positive/negative result combinations for the maximum Legiolert results and the ISO *L. pneumophila* results (left side of the slash) as well as the ISO *Legionella* spp. results (right side of the slash) are shown. The McNemar's test indicated that the methods were not different ($p = 0.919$ for ISO *L. pneumophila* counts and $p = 0.617$ for ISO *Legionella* spp. counts). In Table 12, the McNemar's test outcomes of the

positive/negative result combinations for the best Legiolert results and the ISO *L. pneumophila* results (left side of the slash) as well as the ISO *Legionella* spp. results (right side of the slash) are shown. The McNemar's test indicated that the methods were not different ($p = 0.115$ for ISO *L. pneumophila* counts and $p = 0.409$ for ISO *Legionella* spp. counts). The transformation of results lower than three positive wells or cfu led to a reduction of positive/positive paired data and an increase of negative/negative as well as Legiolert negative/ISO positive combinations. The Legiolert positive/ISO negative number did not increase.

Table 9: McNemar's test for max. or best Legiolert vs. ISO *L. pneumophila* counts.
$\chi 2 = 4.25$, $p = 0.039$.

		ISO *L. pneumophila* counts		
		-	**+**	
Legiolert max. and best	**-**	22	4	26
	+	13	60	73
		35	64	99

Table 10: McNemar's test for max. or best Legiolert vs. ISO *Legionella* spp counts.
$\chi 2 = 0.68$, $p = 0.409$.

		ISO *Legionella* spp. counts		
		-	**+**	
Legiolert max. and best	**-**	19	7	26
	+	11	62	73
		30	69	99

Table 11: McNemar's test for max. Legiolert vs. ISO *L. pneumophila* (left side of the slash) and *Legionella* spp. (right side of the slash) counts.
Results calculated from less than three positive wells or colonies were counted as negative results.
L. pneumophila counts: $\chi 2 = 0.01$, $p = 0.919$ / *Legionella* spp. counts: $\chi 2 = 0.25$, $p = 0.617$.

		ISO counts *L. pneumophila / Legionella* spp.		
		-	**+**	
Legiolert max.	**-**	31 / 29	12 / 14	43 / 43
	+	12 / 11	44/ 45	56 / 56
		43 / 40	66 / 50	99

Table 12: McNemar's test for best Legiolert vs. ISO *L. pneumophila* (left side of the slash) and *Legionella* spp. (right side of the slash) counts.
Results calculated from less than three positive wells or colonies were counted as negative results.
L. pneumophila counts: $\chi 2 = 2.48$, $p = 0.115$ / *Legionella* spp. counts: $\chi 2 = 0.68$, $p = 0.409$.

		ISO counts *L. pneumophila / Legionella* spp.		
		-	**+**	
Legiolert best	**-**	31 / 29	5 / 7	36 / 36
	+	12 / 11	51 / 52	63 / 63
		43 / 40	56 / 59	99

In this study, 10 % of all positive Legiolert wells were tested. In total, 343 wells of the 10 mL trays and 240 of the 1 mL trays. In 330 of the 343 wells of the 10 mL trays, the presence of *L. pneumophila* was confirmed (false-positive rate 3.8 %). In 230 of the 240 wells of the 1 mL trays the presence of *L. pneumophila* was confirmed (false-positive rate 4.2 %). The average false-positive rate for all 783 tested wells amounted to 3.9 %.

The data sets were tested for normal distribution (Kolmogorov-Smirnov test with Lilliefors correction, Shapiro-Wilk test, Anderson-Darling test (60)). None of the data sets were normally distributed. The Wilcoxon signed-rank test (170) was used to test for significant differences between the data sets. The Legiolert max. dataset differed significantly from both the ISO *Legionella* spp. and *L. pneumophila* datasets ($p < 0.05$). No significant difference was recorded for the Legiolert best dataset compared to the *Legionella* spp. or *L. pneumophila* datasets ($p > 0.05$).

After the McNemar's test and the Wilcoxon signed-rank test, the results of both methods were compared according to ISO 17994 (79). Table 13 shows both the max. and the best Legiolert data sets compared to the ISO *L. pneumophila* data set. Both the max. and the best data sets provided significantly higher results for Legiolert because the confidence intervals were located on the positive side outside the 20 % limits (see Table 13 and Figure 10).

Table 13: Comparative analyses of paired data from Legiolert max. and best results with ISO *L. pneumophila* counts.

[a] Half width of the "confidence interval" around the mean relative difference. [b] Value of the relative difference at the lower "confidence limit". [c] Value of the relative difference at the upper "confidence limit".

Data	Number of results	Mean relative difference [%]	Standard deviation [%]	W [a] [%]	X_L [b] [%]	X_U [c] [%]	Outcome
Legiolert max. vs. ISO	77	95.0	251.0	57.2	37.8	152.2	Trial method: higher recovery
Legiolert best. vs. ISO	77	63.5	260.5	59.4	4.2	122.9	Trial method: higher recovery

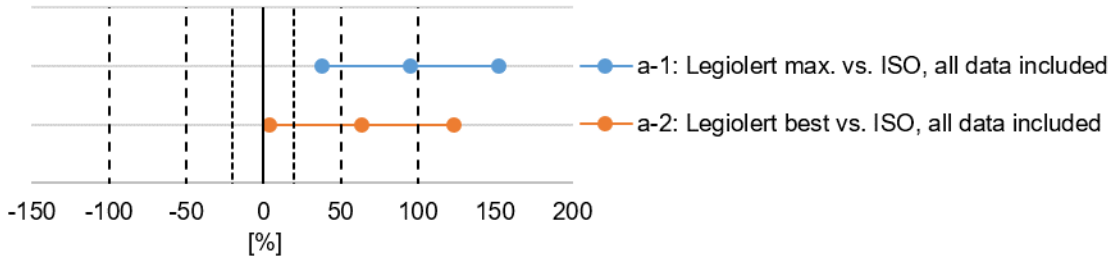


Figure 10: Location of the "confidence intervals" from comparative analyses according to ISO 17994 of paired data from Legiolert and ISO *L. pneumophila* results.

The comparative analyses for the max. and best Legiolert and the ISO *Legionella* spp. data sets are shown in Table 14. Both for the comparison of the max. and the best Legiolert data set with the ISO *Legionella* spp. data set, it was not possible to determine statistically whether the methods are equal or different (see Table 14 a). This is due to the position of the lower limit of the confidence interval in the negative range below the 20 % limit and the upper limit in the positive range above the 20 % limit (see Figure 11 a-1 and a-2).

Table 14: Various comparative analyses according to ISO 17994 of paired data from Legiolert and ISO *Legionella* spp. counts.

[a] Half width of the "confidence interval" around the mean relative difference. [b] Value of the relative difference at the lower "confidence limit". [c] Value of the relative difference at the upper "confidence limit".

Data	Number of results	Mean relative difference [%]	Standard deviation [%]	W [a] [%]	X_L [b] [%]	X_U [c] [%]	Outcome
a) All data included							
Legiolert max. vs. ISO	80	43.1	298.6	66.8	-23.7	109.9	Inconclusive
Legiolert best. vs. ISO	80	12.8	301.6	67.4	-54.6	80.3	Inconclusive
b) Conversion of results calculated from less than three positive wells or colonies to zero							
Legiolert max. vs. ISO	70	43.5	393.3	94.0	-50.5	137.5	Inconclusive
Legiolert best. vs. ISO	70	61.3	334.9	80.1	-18.8	141.3	Inconclusive
c) Legiolert results rounded to two significant digits							
Legiolert max. vs. ISO	80	43.3	298.9	66.8	-23.5	110.2	Inconclusive
Legiolert best. vs. ISO	80	13.0	301.8	67.5	-54.5	80.5	Inconclusive
d) Conversion of results calculated from less than three positive wells or colonies to zero and Legiolert results rounded to two significant digits							
Legiolert max. vs. ISO	70	-41.0	354.0	82.3	-123.3	41.3	Inconclusive
Legiolert best. vs. ISO	70	-24.2	308.4	71.7	-95.9	47.5	Inconclusive
e) Removal of results less than three cfu or mpn							
Legiolert max. vs. ISO	45	44.4	105.6	31.5	12.9	75.9	Trial method: higher recovery
Legiolert best. vs. ISO	52	-7.4	133.7	37.1	-44.5	29.7	Inconclusive*
f) Removal of results less than three cfu or mpn and Legiolert results rounded to two significant digits							
Legiolert max. vs. ISO	45	44.5	105.9	31.6	13.0	76.1	Trial method: higher recovery
Legiolert best. vs. ISO	52	-7.4	133.7	37.1	-44.5	29.7	Inconclusive*
g) 1 mL Legiolert and the 1 mL heat count that gave the final result							
Legiolert vs. ISO	17	-7.5	79.8	38.7	-46.2	31.2	Inconclusive*
h) 1 mL Legiolert and 1 mL heat counts							
Legiolert vs. ISO	30	104.1	211.3	51.6	52.4	155.7	Trial method: higher recovery

By regarding the location of the confidence intervals in Figure 11 a-1 and a-2 it was assumed that the max. Legiolert data set would usually yield significantly higher results than the ISO *Legionella* spp. data set and that the methods were equal when comparing the Legiolert best results with the ISO *Legionella* spp. results. However, the confidence intervals extended over a too wide range for a statistically verified statement.

Thus, for these data sets an attempt was made to reduce the standard deviation and consequently the width of the confidence interval. Results with a very high measurement uncertainty (< 3 positive wells or cfu) were transformed to zero, Legiolert results were rounded to two significant digits and / or only results were considered that were not influenced by a very high measurement uncertainty (see Table 14 b to f).

The conversion of results calculated from less than three positive wells or colonies to zero or rounding the Legiolert results to two significant digits (see Table 14 c) as well as the combination of both (see Table 14 d) was not able to change the ISO 17994 outcome.

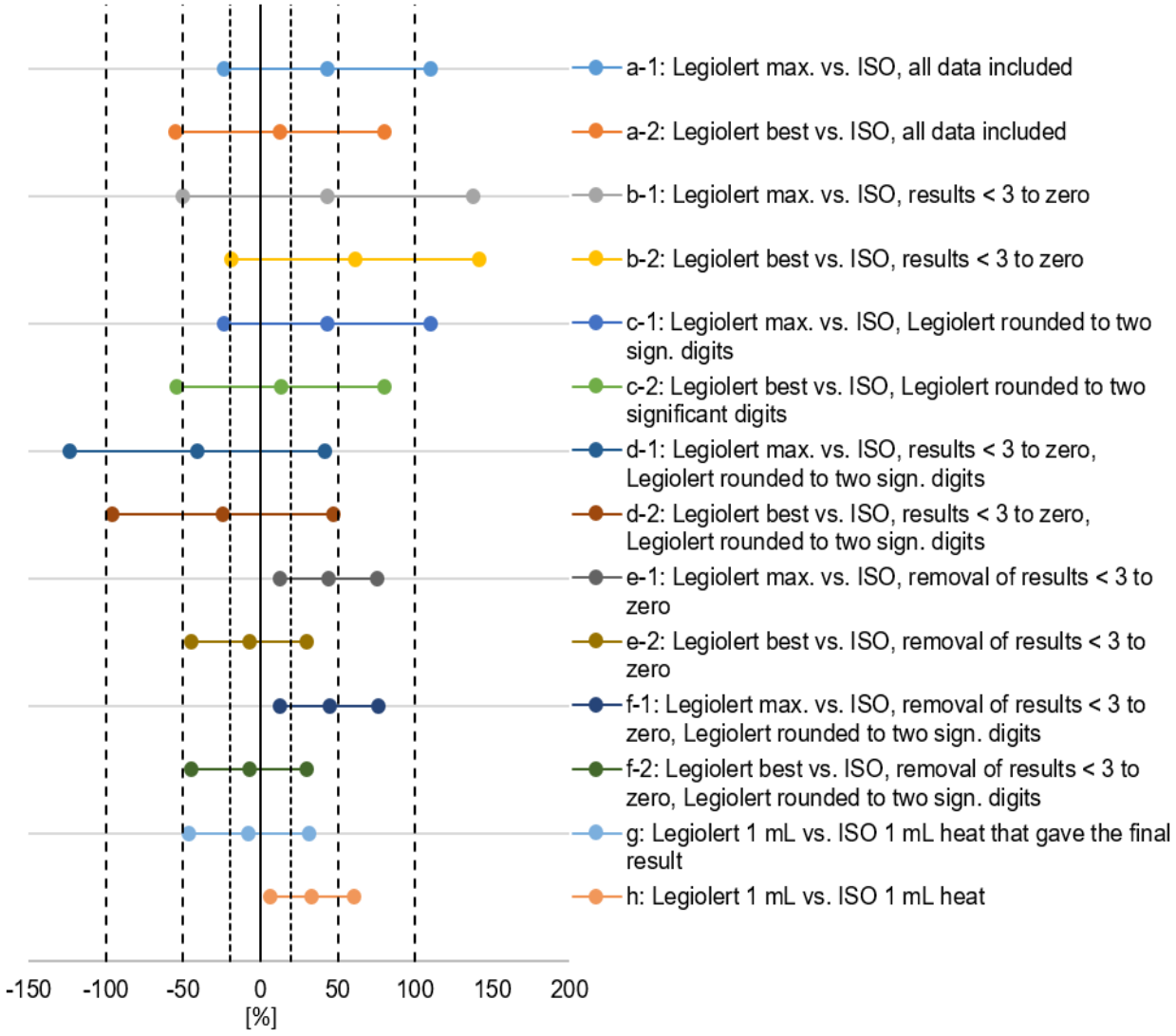


Figure 11: Location of the confidence intervals of relative differences [%] from comparative analyses according to ISO 17994 of paired data from Legiolert and ISO 11731 *Legionella* spp. results.

The removal of results derived from counts lower three positive wells or cfu significantly reduced the standard deviation (see Table 14 e-1, e-2). Thus, it also influenced the width of the confidence intervals to such an extent that the maximum Legiolert data set provided significantly higher results. The corresponding confidence interval of the comparison of the best Legiolert with the ISO *Legionella* spp. values is equally in the negative and positive range, but outside the 20 % limits.

It is doubtful whether it is possible to lie within the 20 % limits under the given circumstances (determination of the final result from different volumes and extrapolation to 100 millilitres). It is therefore reasonable to assume that the methods can be considered as equal if they are within the 50 % limits. In order to exclude the influence of the different volumes and extrapolation in the comparative analysis, Legiolert 1 mL results (mpn/mL) were compared with the ISO results from the 1 mL heat procedure (cfu/mL), which provided the final result (see Table 14 g). In 17 samples, the 1 mL heat treatment step yielded the final result. The outcome is inconclusive, probably due to the small number of samples. Therefore, in a further comparison all 1 mL Legiolert results were compared with the 1 mL heat treatment results (Table 14 h, n = 30). In this case, the Legiolert results were significantly higher. These two analyses were performed to eliminate the influence of the different volumes and extrapolation and to consider how the position of the confidence interval changes to get an impression how the “confidence intervals” change. This analysis is not suitable for final method comparison.

For the cooling tower operator the comparison of the legionellae concentration in the sample with the test and action values of the German 42nd Ordinance Implementing the Federal Immission Control Act (1) is indispensable. Table 15 to Table 18 show the classification of the data pairs regarding the test and action values. The Mc Nemar’s test was performed for the sum of data pairs where one method’s result exceeded a higher test/action value than the other.

For the classification of Legiolert max. results and ISO *L. pneumophila* results 74 of 99 samples (74.7 %) were in the same test/action value range of 42nd BImSchV (see Table 15). Legiolert max. results exceeded a higher test/action value in 22 samples (22.2 %). Vice versa ISO *L. pneumophila* results exceeded a higher test/action value in three samples (3.0 %). The Mc Nemar’s test indicated that the methods are different (p = 0.001). Regarding the number of samples exceeding a higher test/action value, it was concluded that Legiolert max. results exceeded significantly more often a higher test/action value than ISO *L. pneumophila* ones. For the classification of Legiolert best results and ISO *L. pneumophila* results 67 of 99 samples (67.7 %) lay in the same test/action value range of 42nd BImSchV (see Table 16). Legiolert best results exceeded a higher test/action value in 19 samples (19.2 %). Vice versa, ISO *L. pneumophila* results exceeded a higher test/action value in 13 samples (13.1 %). The McNemar’s

test indicated that the methods are different ($p = 1.95 * 10^{-5}$). Regarding the number of samples exceeding a higher test/action value, it was concluded that Legiolert best results exceeded significantly more often a higher test/action value than ISO *L. pneumophila* ones.

Table 15: Classification of Legiolert max. and ISO *L. pneumophila* results acc. to 42nd BImSchV. Mc Nemar's test: $\chi^2 = 10.66$, p-value = 0.001.

		ISO 11731 *L. pneumophila* results				
		≤ Test Value 1	> Test Value 1	> Test Value 2	> Action Value	
Legiolert max.	≤ Test Value 1	**35**	2	0	0	37
	> Test Value 1	6	**14**	1	0	21
	> Test Value 2	4	10	**17**	0	31
	> Action Value	1	0	1	**8**	10
		46	26	19	8	99

Table 16: Classification of Legiolert best and ISO *L. pneumophila* results acc. to 42nd BImSchV. Mc Nemar's test: $\chi^2 = 18.23$, p-value = $1.95 * 10^{-5}$.

		ISO 11731 *L. pneumophila* results				
		≤ Test Value 1	> Test Value 1	> Test Value 2	> Action Value	
Legiolert best	≤ Test Value 1	**36**	8	0	0	44
	> Test Value 1	6	**10**	5	0	21
	> Test Value 2	3	8	**13**	0	24
	> Action Value	1	0	1	**8**	10
		46	26	19	8	99

Table 17: Classification of Legiolert max. and ISO *Legionella* spp. results acc. to 42nd BImSchV. Mc Nemar's test: $\chi 2 = 5.58$, p-value = 0.018.

		ISO 11731 *Legionella* spp. results				
		≤ Test Value 1	> Test Value 1	> Test Value 2	> Action Value	
Legiolert max.	≤ Test Value 1	**32**	3	2	0	37
	> Test Value 1	4	**14**	2	1	21
	> Test Value 2	4	10	**17**	0	31
	> Action Value	1	0	1	**8**	10
		41	27	22	9	99

Table 18: Classification of Legiolert best and ISO *Legionella* spp. results acc. to 42nd BImSchV. Mc Nemar's test: $\chi^2 = 0.007$, p-value = 0.934.

		ISO 11731 *Legionella* spp. results				
		≤ Test Value 1	> Test Value 1	> Test Value 2	> Action Value	
Legiolert best	≤ Test Value 1	**32**	9	2	1	44
	> Test Value 1	5	**10**	6	0	21
	> Test Value 2	3	8	**13**	0	24
	> Action Value	1	0	1	**8**	10
		41	27	22	9	99

For the classification of Legiolert max. results and ISO *Legionella* spp. results 71 of 99 samples (71.7 %) were in the same test/action value range of 42nd BImSchV (see Table 17). Legiolert max. results exceeded a higher test/action value in 20 samples (20.2 %). Vice versa ISO *Legionella* spp. results exceeded a higher test/action value in 13 samples (8.1 %). The Mc Nemar's test indicated that the methods are different (p = 0.018). Regarding the number of samples exceeding a higher test/action value, it was concluded that Legiolert max. results exceeded significantly more often a higher test/action value than ISO *Legionella* spp. ones. For the classification of Legiolert best results and ISO *Legionella* spp. results 63 of 99 samples (63.6 %) were in the same test/action value range of 42nd BImSchV (see Table 18). Each method exceeded a higher test/action value in 18 samples (18.2 %). The Mc Nemar's test indicated that the methods are not different (p = 0.934).

The measurement uncertainty of the final results is shown in Figure 12. With the ISO method, about 75 % of the results were influenced by a low measurement uncertainty. Approximately 10 % of the results were each influenced by a high or very high measurement uncertainty according to ISO 8199 and the recommendation of the Federal Environment Agency (81, 155). About half of the results obtained with Legiolert were influenced by a low measurement uncertainty, about one third of the results by a high measurement uncertainty and 10 to a maximum of 25 % of the results by a very high measurement uncertainty. The best results included the lowest number of results with a very high measurement uncertainty. In comparison to the max. results, the best results included seven results (10 %) less influenced by a very high measurement uncertainty.

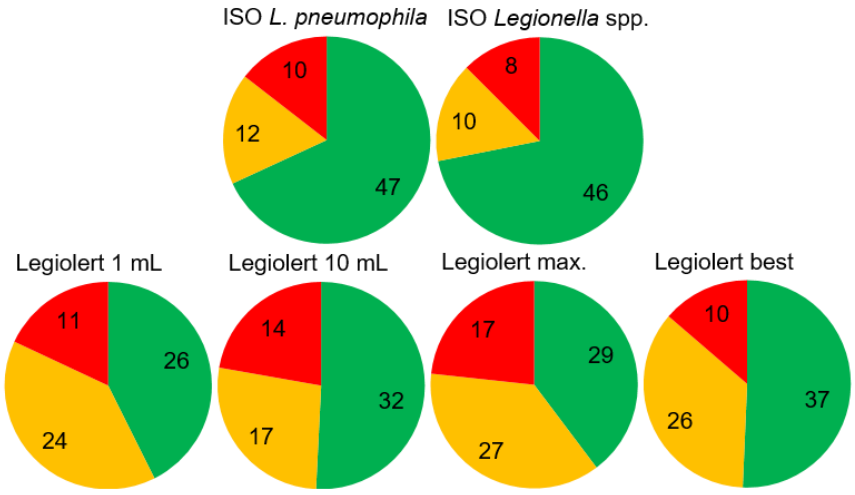


Figure 12: Measurement uncertainty (MU) of ISO and Legiolert counts.
Green: cfu or positive wells ≥ 10, low MU; orange: 10 > cfu or positive wells ≥ 3, high MU; red: cfu or positive wells < 3, very high MU

3.2 Retrospective analyses of microbial concentrations in cooling tower samples

In this section, the results of the retrospective analyses and their possible correlation with the process parameters redox potential and water temperature are shown for the four cooling towers.

In the second part of this section a comparison of heterotrophic plate counts at the two incubation temperatures was performed. The comparison included 2,868 industrial samples from the Laboratory for Technical Hygiene at IHPH.

3.2.1 Retrospective analysis of process parameters and microbiological concentrations in four cooling towers

In this section, the results of the four cooling towers of the retrospective analyses from 2012 to 2018 are shown for the legionellae concentrations and their possible correlation with the process parameters redox potential and water temperature. Furthermore, the retrospective analyses of HPC and their possible correlation with the process parameter water temperature included data from 2017 to 2018. The retrospective analyses of HPC and their possible correlation with the process parameter redox potential included data from 2017 to 2019.

This section contains five subsections. In four subsections, the results of the retrospective analysis for each cooling tower are described. For each cooling tower the microbiological data of the retrospective observation period are presented and visualized via figures. The first figure shows the course of the legionellae concentrations during the seven-year monitoring period. The second figure is a circular diagram and shows the number of the detected *Legionella species* in the 84 samples. In addition to the circular diagrams, Table 31 in section 3.2.1.5 lists how the strains detected by the Latex test kit were distributed in each of the 84 samples. The third figure shows the course of the HPC. The fourth figure shows the boxplots of the data sets. A table follows this fourth figure containing the number of calculated outliers. The next table includes the results of the regression analyses of the microbiological data and the process parameters. If the correlation was stastically significant ($>|0.3|$ (104)) scatter plots of the corresponding data are shown. In the fifth subsection, the legionellae concentrations of the four cooling towers were retrospectively subdivided according to the test and action values of the 42nd BImSchV (1).

For the analysis of the microbiological parameters as a function of the process parameters,

- the **unchanged** microbiological results and
- the **log**$_{10}$-transformed results (log)

were considered.

In the original data, the final result for samples without target organism detection was expressed according to the detection limit depending on which test volumes was clearly evaluable regarding the accompanying flora. In figures and calculations of this study part, missing detection was given as “zero” results, or as “one” in log-transformed charts.

The general distribution of the legionellae concentrations and HPC are traceable by the box plots. They serve as a supplement to the concentration curves and allowed to determine outliers. The grey box shows 50 % of the results of the investigated samples. The lower edge of the box marks the 25 % quartile, the upper edge the 75 % quartile. To detect outliers in the microbiological data sets, the upper and lower quartiles of the data were determined. Microbiological values that exceeded or dropped below the upper or lower quartile by 1.5 times the interquartile range were identified as outliers (62). “One” must be regarded as the minimum (corresponding to a result below the detection limit), since the calculated minima in the negative value range were unrealistic. The retrospective analyses were carried out in order to generate a tool that allows predictions of the legionellae concentration or the HPC due to changes in the water temperature or the redox potential. Because of this, it is not reasonable to remove the outliers from the data set. Due to the good maintenance measures of the investigated cooling towers, these outliers are essential for the creation of a "prediction tool". By determining the outliers, it was clarified how often events led to a particular increase in microbiology in the cooling towers. For each cooling tower, only the unchanged data sets are shown as box plots. The black lines mark the area of the 1.5 times interquartile range of the upper or lower quartile. The red line marks the area where outliers were located. No box plots are shown for the log-transformed data sets, even if the number of outliers was different. The outliers of the respective microbiological data sets are listed for each cooling tower in the table below the figure with the box plots.

The redox potentials were considered over a certain observation period. Three observation periods each (five, seven and ten days) were investigated. To obtain a value against which the microbiological results were plotted,

1. the number of biocide dosages during the observation period before sampling,
2. the number of biocide dosages during the observation period after sampling,
3. the sum of biocide dosages during the observation period before and after sampling,
4. the ratio of the number of biocide dosages (during the observation period after / before sampling),
5. the averaged redox potential during the observation period before sampling and
6. the period of measurements below the selected redox potential threshold during the observation period before sampling

were calculated. The number of biocide dosages was determined by looking at the successive hourly-recorded redox potential values. If the subsequent value was higher than the previous value and above 400 mV, respectively 350 mV in Cooling Tower 2, it was assumed that biocide dosing had taken place. The biocide dosages were summed over the selected period.

The water temperatures of the cooling water flow to the exchanger were also observed over a selected observation period. To obtain a value against which the microbiological result were plotted,

1. the mean temperature within the observation period before sampling,
2. the minimum temperature within the observation period before sampling,
3. the maximum temperature within the observation period before sampling and
4. the period of measurements above the threshold temperature within the observation period before sampling

were calculated.

The microbiological *Legionella* and HPC data and the different calculations of the process parameters are shown in the annex.

The tables on pages 70, 75, 82, 86 show the results with the best correlations of the regression analyses for the modified redox potential and water temperature data sets with the microbiological data sets.

In the column "Data sets" the combinations of the microbiological and process parameter data sets are listed, which provided the highest Pearson correlation r. The first part of the combination denotes the microbiological data set (unchanged = the cfu per volume results, log = the log-transformed cfu per volume results). An underscore separates the first part of the code from the second one. The second part of the code indicates which modified process parameter data set is involved (1 - 6 for the redox potential data sets or 1 - 4 for the water temperature data sets according to list on pages 65 and 66). For example “log_3” refers to the log-transformed microbiological data set in combination with the modified process parameter data set number three. The column "Correlation r (Pearson)" shows the value of the Pearson correlation. The column "Formula" shows the regression line if the modulus of the Pearson correlation was ≥ 0.3. In this case, the correlation is statistically significant ($p \leq 0.05\,\%$). The corresponding plots with the best correlation for each microbiological parameter are shown including the regression line function and the coefficient of determination (R^2, the squared Pearson correlation value r).

3.2.1.1 Cooling Tower 1

The legionellae concentrations in 84 water samples from Cooling Tower 1 fluctuated only slightly as shown in Figure 13. Frequently, in fact in 31 samples, no Legionellae were detected. There is no indication that Legionellae were present in this cooling tower in increased quantities depending on the season. Rather, the *Legionella* spp. concentration can be regarded as consistently low between 10 and 100 cfu/100 mL. As shown in Figure 35, 45 samples were located in this range. In eight samples, the concentration ranged between 100 and 1,000 cfu/100 mL. In six samples, *L. pneumophila* serogroup 1 was detected. In 52 samples, *L. pneumophila* strains of the serogroup range 2-14 were determined. In one sample, a *Legionella species* strain, which did not show a positive reaction with any of the Latex test antisera, was detected. In six samples, two different *Legionella* strains were identified at the same time. An additional biocide dosing was carried out on August 17th 2012 with peracetic acid. In Figure 13, a red line marks the special dosage. A decrease of the *Legionella* spp. concentration was observed during two months after the dosage. As the legionellae concentration was not extraordinarily high before dosing, peracetic acid was probably dosed due to a high algae load in the cooling tower, the internals and/or in the pipeline network.

The HPC from 2017 to 2019 are shown in Figure 15.The blue crosses belong to the results at 22 °C, the red ones to those at 36 °C. In total, 41 HPC data pairs were included in the redox potential analysis and 23 HPC data pairs were included in the water temperature analysis. Considering all 41 investigations, 29 results were in the same range when applying the internal laboratory rule that defines microbiological results as equivalent if they differ less than a half log level. Ten samples had higher HPC at 22 °C, two at 36 °C. Most of the values measured ranged between 10^2 and 10^3 cfu/mL (see Figure 15). While it seems that HPC increased significantly from April to July 2017, no seasonal increases were recorded in early summer in 2018 and 2019. A continuous increase in HPC with the highest peak from October onwards occurred after the deactivation of biocide dosing in October 2019.

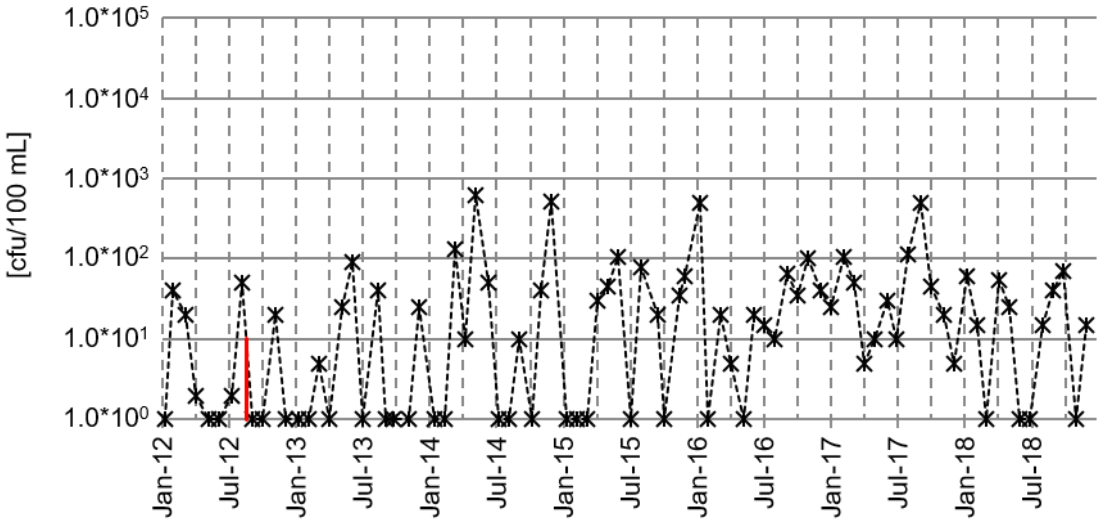


Figure 13: CT1 - *Legionella* spp. concentrations from 2012 to 2018.
Red line: special peracetic acid biocide dosage.

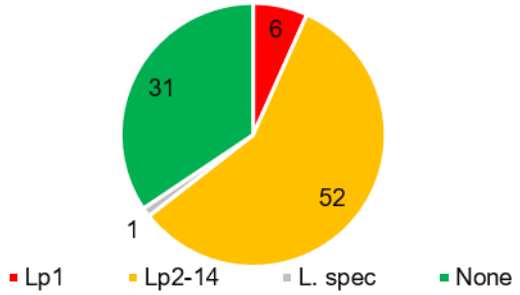


Figure 14: CT1 – Distribution of *Legionella* strains in 84 water samples from 2012 to 2018.

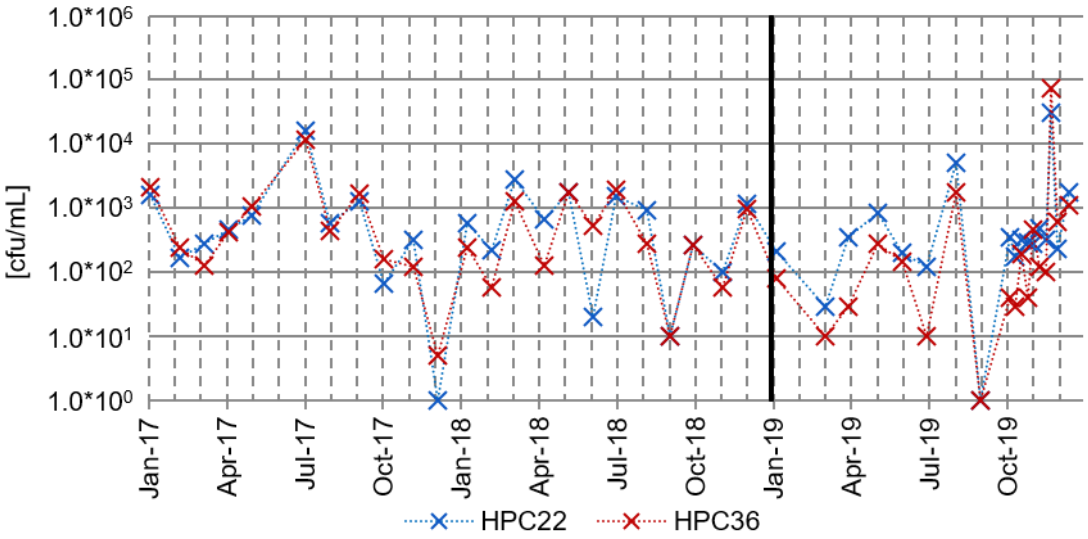


Figure 15: CT1 - HPC from 2017 to 2019.

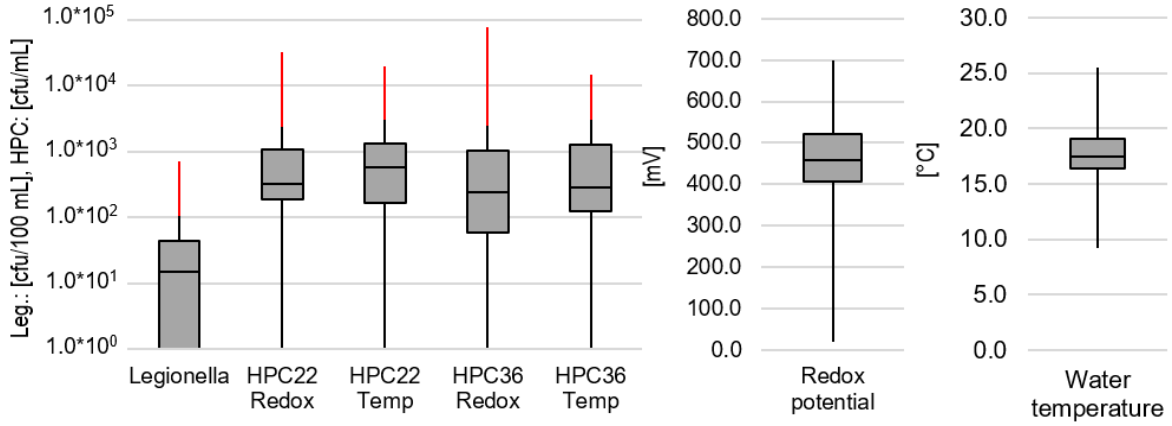


Figure 16: CT1 - Boxplots of the different data sets.
Unchanged microbiological data ("one" corresponds to a result below the detection limit), process parameter data from 2012 to 2018 (water temperature data) and 2012 to 2019 (redox potential data).

It was described above that in the majority of the samples no legionellae or concentrations below 100 cfu/100 mL were detected. As shown in the boxplot in Figure 16, in fact, 75 % of the results were below 45 cfu/100 mL and the median was 15 cfu/100 mL. The highest concentration determined is 610 cfu/100 mL. The red range contains the six outliers as listed in Table 19 that counted 108 cfu/100 mL or more.

The HPC 22 °C | 36 °C data sets used for the regression analyses with the redox potential data ranged from below the detection limit ("one") to 30,240 | 73,200 cfu/mL[1]. The medians were 320 | 240 cfu/mL. The lower quartile amounted to 190 | 60 cfu/mL and the upper quartile amounted to 1,065 | 1,030 cfu/mL. For HPC at 22 C, four values were higher than 2,378 cfu/mL. For HPC at 36 °C, two values were higher than 2,485 cfu/mL and determined as outliers.

The HPC 22 °C | 36 °C data sets used for the regression analyses with the water temperature data ranged from below the detection limit ("one") to 16,400 | 11,800 cfu/mL. The medians were 590 | 280 cfu/mL. Of the HPC data 50 % ranged from 169 to 1.300 | from126 to 1.290 cfu/mL. Each one value higher than 2,997 | 3,036 cfu/mL was determined as an outlier.

The outliers of the microbiological data sets are listed in Table 19. Due to the removal of implausible data, no outliers existed for the process parameter data sets. The value range of the redox potential data (2012 to 2019) extended from 20 to 700 mV. The median amounted to 457 mV. Of the redox potential data, 50 % were localized between 408 and 521 mV. The value range of the water temperature data (2012 to 2018) extended from 9.2 to 25.5 °C. The median was 17.5 °C. Of the water temperature data, 50 % were localized between 16.4 and 19.1 °C.

[1] The left value of the dividing line belongs to the 22 °C-count, the right value to the 36 °C-count.

Table 19: CT1 – Number of outliers of the microbiological data.

Dataset	LEG	log LEG	HPC22	log HPC22	HPC36	log HPC36
Redox potential	2012-2018		2017-2019		2017-2019	
	6	0	4	5	2	1
Water temperature	2012-2018		2017-2018		2017-2018	
	6	0	1	1	1	0

The regression analyses of (the log-transformed) *Legionella* spp. and 22 °C·HPC values in dependence of redox potential data (see list on page 65) provided moderate correlations (< -0.3) in three cases (see Table 20). Scatter plots of the regression analyses with the highest correlations, considering the modulus of the correlation, are shown in the scatter plots in Figure 17. The correlations of the HPC at 36 °C values with the different redox potential data were less than 0.3, so no scatter plot was generated

Table 20: CT1 - Results of the regression analyses of the microbiological and redox potential data.

Parameter	Data sets	Correlation r (Pearson)	Assessment / Formula
Observation period: 5 days, redox potential threshold: 400 mV			
Legionella	**log _1**	**-0.353**	**$y_{log10\_Lspp} = -0.289x_{Redox\_1} + 1.4959$**
HPC 22 °C	log _2	0.257	Correlation not significant
HPC 36 °C	log _3	0.208	Correlation not significant
Observation period: 7 days, redox potential threshold: 400 mV			
Legionella	log _3	-0.321	$y_{log10\_Lspp} = -1.0109x_{Redox\_3} + 1.5337$
HPC 22 °C	**log _4**	**-0.302**	**$y_{log10\_HPC22} = -0.0572x_{Redox\_4} + 2.6476$**
HPC 36 °C	log _2	0.295	Correlation not significant
Observation period: 10 days, redox potential threshold: 400 mV			
Legionella	log _3	-0.293	Correlation not significant
HPC 22 °C	unchanged _2	0.295	Correlation not significant
HPC 36 °C	log _2	0.279	Correlation not significant

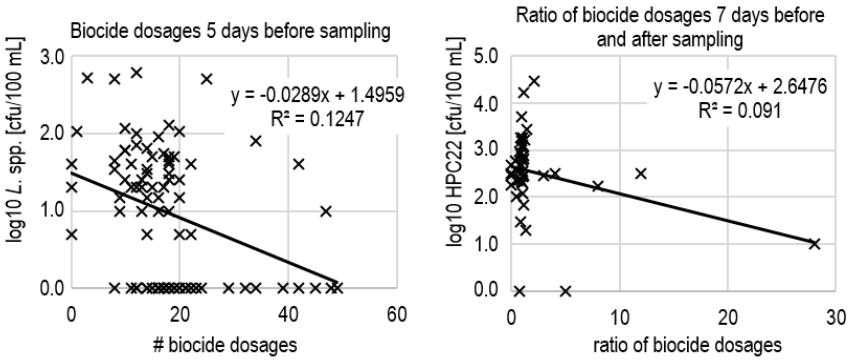


Figure 17: CT1 - Regression lines of the microbiological concentrations and modified data derived from the redox potential data.

The highest correlation of *Legionella* spp. concentrations depending on the redox potential was obtained for the combination of log-transformed *Legionella* concentrations and the number of biocide dosages within five days before sampling. In the left chart of Figure 17, a circular inner cloud of points is visible, surrounded by an outer ring of data. The regression line has a plausible negative slope indicating that an increasing number of biocide dosages is associated

with a decreased *Legionella* spp. concentration. The highest Legionellae concentrations were associated with a number of dosages between zero and 30. No *Legionella* spp. detection occurred almost across the whole range of biocide dosages during five days before sampling. The maximum *Legionella* spp. concentration determinable with the regression line is approximately 30 cfu/100 mL, if no dosages take place within five days before sampling.

The highest correlation of HPC at 22 °C values depending on the redox potential was obtained for the combination of log-transformed counts and the ratio of biocide dosages within seven days after and before sampling (after/before). In the right chart of Figure 17, an elongated point cloud extends over the entire ordinates range at a ratio smaller than five on the abscissa. Six data with ratios of biocides higher than the logarithmic value of five" strongly influenced the regression line. Higher ratios of biocide dosages seven days after to before sampling tend to be associated with lower HPC at 22 °C. The maximum HPC at 22 °C determinable with the regression line for an after/before-ratio of zero (i. e. no dosages take place during seven days after sampling) is approximately 450 cfu/mL.

The regression analyses of the log-transformed HPC values at 22 °C incubation temperature in dependence of the minimum water temperature data (see list on page 66) provided moderate correlations (< -0.3) regarding five, seven and ten days before sampling (see Table 21).

Table 21: CT1 - Results of the regression analyses of the microbiological and water temperature data.

Parameter	Datasets	Correlation r (Pearson)	Assessment / Formula
Observation period: 5 days, water temperature threshold 20 °C			
Legionella	unchanged _2	0.003	Correlation not significant
HPC 22 °C	log _2	-0.323	$y_{log10\_HPC22} = -0.1658x_{Temp\_2} + 5.1603$
HPC 36 °C	unchanged _4	0.261	Correlation not significant
Observation period: 7 days, water temperature threshold 20 °C			
Legionella	unchanged _3	0.045	Correlation not significant
HPC 22 °C	**log _2**	**-0.328**	$\mathbf{y_{log10\_HPC22} = -0.1676x_{Temp\_2} + 5.1869}$
HPC 36 °C	unchanged _3	0.241	Correlation not significant
Observation period: 10 days, water temperature threshold 20 °C			
Legionella	log _3	0.071	Correlation not significant
HPC 22 °C	log _2	-0.302	$y_{log10\_HPC22} = -0.0192x_{Temp\_2} + 2.9274$
HPC 36 °C	unchanged _4	0.287	Correlation not significant

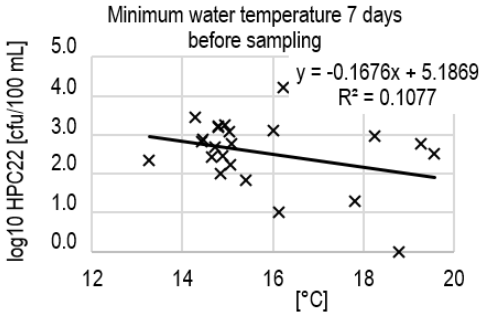


Figure 18: CT1 - Regression line of the log10 HPC at 22 °C and the minimum water temperature seven days before sampling.

The highest correlation of HPC at 22 °C values was obtained for the combination of log-transformed counts and the minimum water temperature during seven days before sampling. Figure 18 shows a point cloud distributed over a large area along the regression line with a concentrated occurrence of data between 14 and 16 °C minimum water temperature within seven days before sampling. The slope of the regression line is negative indicating that a higher minimum water temperature within seven days is associated with decreased HPC at 22 °C. The maximum HPC at 22 °C for a plausible minimum temperature of 4 °C within seven days before sampling calculated with the regression line is approximately $3.3 * 10^5$ cfu/mL.

3.2.1.2 Cooling Tower 2

As mentioned in subsection 1.4.2, microbial concentrations before 2018 were obtained from the other cooling tower of the cooling system.

In Cooling Tower 2, legionellae were detected in 82 of 84 water samples during the observation period from 2012 to 2018. In 43 samples, concentrations between 100 and 1,000 cfu/100 mL, in 19 samples between 1,000 and 10,000 cfu/100 mL and in two samples concentrations > 10,000 cfu/100 mL were detected (see Figure 35). Looking at the curve in Figure 19, a trend can be assumed that the legionellae concentration dropped in the autumn and winter months and rised from spring to summer. In the seven years of observation, six special biocide dosages with peracetic acid were carried out. With the exception of the special dosage in April 2018, the peracetic acid was presumably not dosed due to high legionellae concentrations, as the previous concentrations were not extraordinarily high. Probably the special dosages were carried out due to algae growth. However, in five cases the special dosages have led to a reduction in the concentration of *Legionella* spp., which lasted for a few months. In contrast to these five dosages, it was noticeable in 2018 that immediately after the special dosage in February, legionellae were detected in a concentration of 40 cfu/100 mL similar to the concentration of 20 cfu/100 mL in the previous sample. In the following sample no *Legionella* spp. were detected. However, the effect of the dosage did not last long and the concentration rapidly increased to 13,000 cfu/100 mL within one month. The subsequent special dosage initially led to a decrease of the legionellae concentration and then again to an repeated increase to 2.700 cfu/100 mL in September.

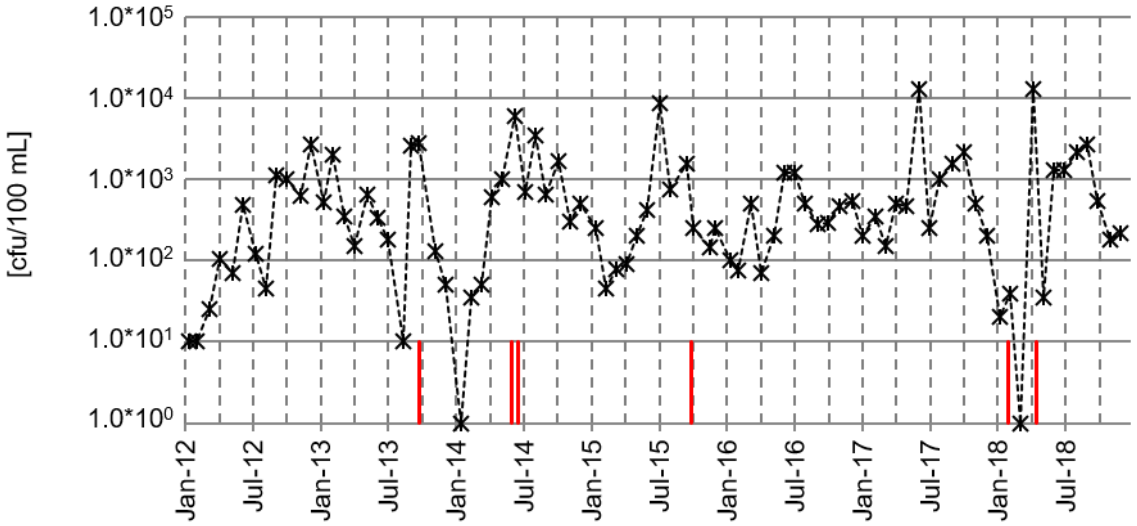


Figure 19: CT2 - *Legionella* spp. concentrations from 2012 to 2018.
Red lines: special peracetic acid biocide dosages.

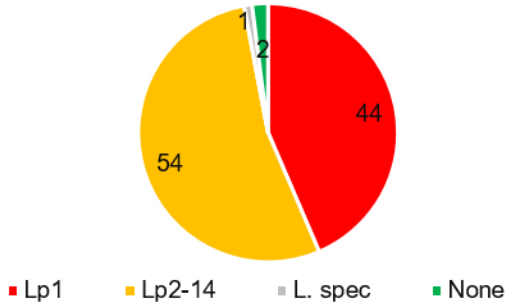


Figure 20: CT2 – Distribution of *Legionella* strains in 84 samples from 2012 to 2018.

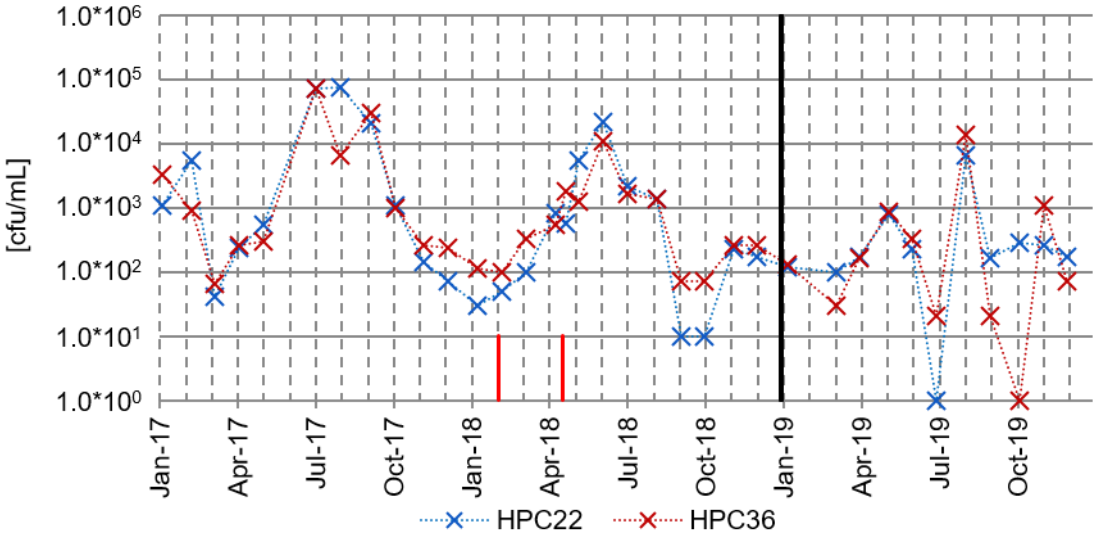


Figure 21: CT2 - HPC from 2017 to 2019.
Red lines: special peracetic acid biocide dosages.

From September 2018 onwards, a decrease was recorded again, probably due to seasonal factors. In the 82 samples, 44 times *L. pneumophila* serogroup 1, 54 times *L. pneumophila* of the serogroup range 2 - 14, and one time a *Legionella species* strain that did not show a positive reaction in the Latex test were identified (see Figure 20). In 17 samples, two *Legionella* strains were recorded at the same time.

The course of the HPC curves was very similar for both incubation temperatures (see Figure 21). Considering all 35 investigations, 22 results were in the same range when using the internal laboratory rule that defines microbiological results as equivalent if they differ less than a half log level. Most concentrations were located in the range of 10^2 to 10^3 cfu/mL. Six samples had higher HPC at 22 °C, seven at 36 C. The HPC data sets included 35 data pairs for the regression analyses with the redox potential data and 24 data pairs for the regression analyses with the water temperature data. For the years 2017 and 2018, a trend was observed that HPC increased in the spring and summer months and decreased in the autumn and winter months. The two special biocide dosages did not have any effect on the HPC, but did not seem to have been implemented because of them. The increase in HPC in 2019 was not as steady as in the other two years, but was characterized by a fluctuating course during the summer months. However, concentrations similar to those of the previous year were reached in July.

The box plots in Figure 22 show the unchanged microbiological data sets and the process parameter data sets. The determined legionellae concentrations ranged from below the detection limit ("one") to 13,000 cfu/100 mL. 50 % of the values varied between 106 and 1,008 cfu/100 mL. Nine results above 2,359 cfu/100 mL were determined as outliers.

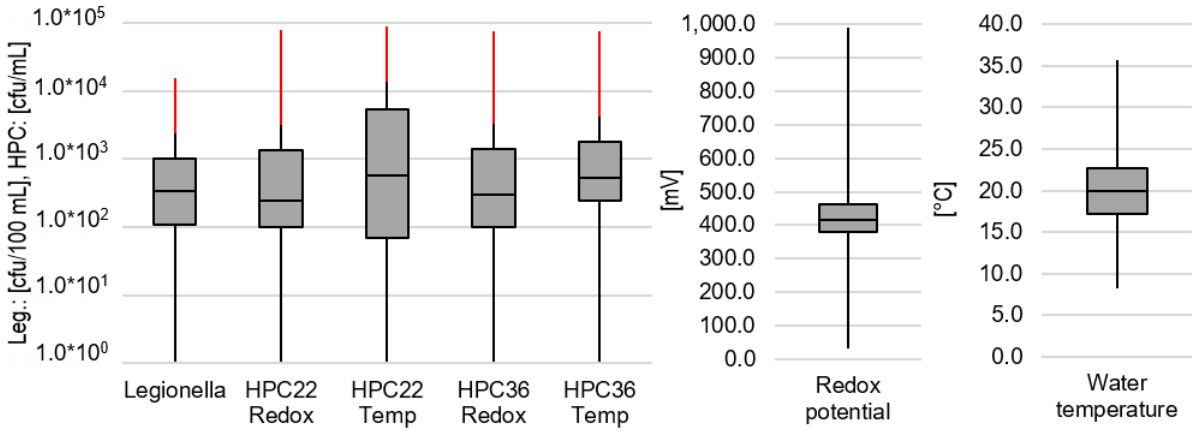


Figure 22: CT2 - Boxplots of the different data sets.
Unchanged microbiological data ("one" corresponds to a result below the detection limit), process parameter data from 2012 to 2018 (water temperature data) and 2012 to 2019 (redox potential data).

The HPC 22 °C | 36 °C data sets used for the regression analyses with the redox potential data ranged from below the detection limit ("one") to 75,600 | 70,200 cfu/mL. The medians were 240 | 300 cfu/mL. Of the HPC results, 50 % ranged from 100 to 1,340 | from 100 to 1,380 cfu/mL.

For HPC at 22 °C, seven values were higher than 3,200 cfu/mL. For HPC at 36 °C, five values were higher than 3,300 cfu/mL. These results were determined as outliers.

The HPC 22 °C | 36 °C data sets used for the regression analyses with the water temperature data ranged from below the detection limit ("one") to 75,600 | 70,200 cfu/mL. The medians were 570 | 530 cfu/mL. Of the HPC, 50 % ranged between 70 and 5,400 | 240 and 1,800 cfu/mL. Each four values were higher than 13,395 | 4,140 cfu/mL and determined as outliers.

The outliers of the microbiological data sets are listed in Table 22. Due to the removal of implausible data, no outliers existed for the process parameter data sets. The value range of the redox potential data (2012 to 2019) extended from 31 to 990 mV. The median amounted to 415 mV. Of the data, 50 % were localized between 380 and 461 mV. The value range of the water temperature data (2012 to 2018) extended from 8.2 to 35.6 °C. The median was 20.0 °C, the lower quartile amounted to 17.2 °C and the upper quartile to 22.7°C.

Table 22: CT2 – Number of outliers of the microbiological data.

Dataset	LEG	log LEG	HPC22	log HPC22	HPC36	log HPC36
Redox potential	2012-2018		2017-2019		2017-2019	
	9	2	7	3	5	1
Water temperature	2012-2018		2017-2018		2017-2018	
	9	2	4	0	4	1

The regression analyses of the microbiological data from Cooling Tower 2 with the modified redox potential data showed Pearson correlations higher than 0.3 for HPC at both incubation temperatures (see Table 23).

For HPC at 22 °C, the best correlations were obtained for the modified redox potential data sets regarding the number of biocide dosages of the three observation periods (five, seven, ten days) before and after sampling. The highest correlation amounted to $r = 0.452$ for the observation period of five days and the corresponding regression line is shown in the left chart of Figure 23. The regression line has a positive slope indicating that increasing numbers of biocide dosages are associated with increased HPC at 22 °C. Most data points extended over the entire abscissa at very low HPC 22 °C values. The two data points in the upper right corner strongly influenced the course of the regression line. Considering a plausible very high number of 100 biocide dosages five days before and after sampling a maximum HPC at 22 °C of $2.9 * 10^4$ cfu/mL is calculable by the regression line. The HPC 22 °C value obtains a positive sign for a number of biocide dosages of 20 or more.

Table 23: CT2 - Results of the regression analyses of the microbiological and redox potential data.

Parameter	Datasets	Correlation r (Pearson)	Assessment / Formula
Observation period: 5 days, redox potential threshold: 350 mV			
Legionella	unchanged _6	0.283	Correlation not significant
HPC 22 °C	**unchanged _3**	**0.452**	**$y_{HPC22} = 394x_{Redox\_3} - 10{,}687$**
HPC 36 °C	log _5	0.327	$y_{log10\_HPC36} = 0.0051x_{Redox\_5} + 0.5084$
Observation period: 7 days, redox potential threshold: 350 mV			
Legionella	unchanged _6	0.234	Correlation not significant
HPC 22 °C	unchanged _3	0.400	$y_{HPC22} = 257.71x_{Redox\_3} - 9{,}569$
HPC 36 °C	log _5	0.342	$y_{log10\_HPC36} = 0.0054x_{Redox\_5} + 0.4023$
Observation period: 10 days, redox potential threshold: 350 mV			
Legionella	unchanged _6	0.206	Correlation not significant
HPC 22 °C	unchanged _3	0.373	$y_{HPC22} = 169.03x_{Redox\_3} - 85945$
HPC 36 °C	**log _5**	**0.365**	**$y_{log10\_HPC36} = 0.006x_{Redox\_5} + 0.1376$**

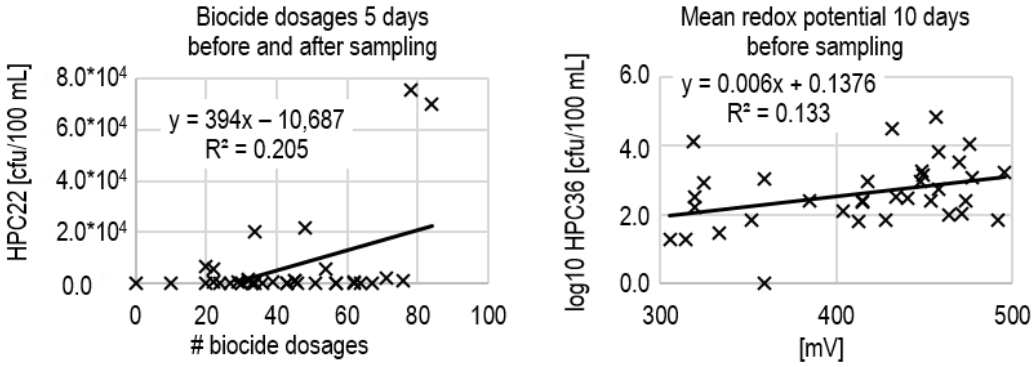


Figure 23: CT2 - Regression lines of microbiological concentrations and modified data derived from the redox potential data.

For log-transformed HPC at 36 °C, the combination with the averaged redox potential values five, seven and ten days before sampling showed the best correlations. The highest correlation amounted to r = 0.365 for the ten days observation period and the corresponding regression line is shown in the right chart of Figure 23. The regression line has a positive slope indicating that increased means of the redox potential values were associated with increased HPC at 36 °C. The data points were widely and rather evenly distributed around the regression line. With a plausible very high averaged mean of 550 mV a maximum HPC at 36 °C of 5,000 cfu/mL is calculable by the regression line. The Pearson correlation of *Legionella* spp. data with the six redox potential data sets was always below 0.3.

The regression analyses of all microbiological data in combination with modified water temperature data sets provided Pearson correlation values higher than 0.5 indicating a large strength of association (see Table 24). For *Legionella* spp. the maximum correlation (r = 0.581) was obtained for the combination of the log-transformed *Legionella* spp. data with the data set of the maximum water temperatures within seven days before sampling. The corresponding regression line is shown in the left chart of Figure 24. The regression line has a positive slope

indicating that an increasing maximum water temperature was associated with increased legionellae concentrations. The data points flank the regression line equally. With a very high maximum water temperature of 35 °C, which is plausible for Cooling Tower 2, a maximum *Legionella* spp. concentration of $1.0 * 10^4$ cfu/100 mL was calculated. Above a maximum water temperature of 5.7 °C, legionellae concentrations receive a positibve sign.

Table 24: CT2 - Results of the regression analyses of the microbiological and water temperature data.

Parameter	Datasets	Correlation r (Pearson)	Assessment / Formula
Observation period: 5 days, water temperature threshold 20 °C			
Legionella	log _3	0.577	$y_{log10\_Lspp} = 0.129x_{Temp\_3} - 0.5535$
HPC 22 °C	unchanged _2	0.552	$y_{HPC22} = 3{,}745x_{Temp\_2} - 52{,}507$
HPC 36 °C	log _2	0.488	$y_{log10\_HPC36} = 0.1316x_{Temp\_2} + 0.7179$
Observation period: 7 days, water temperature threshold 20 °C			
Legionella	**log _3**	**0.581**	$\mathbf{y_{log10\_Lspp} = 0.1352x_{Temp\_3} - 0.7639}$
HPC 22 °C	unchanged _2	0.557	$y_{HPC22} = 3{,}620x_{Temp\_2} - 49{,}463$
HPC 36 °C	log _1	0.507	$y_{log10\_HPC36} = 0.1329x_{Temp\_1} + 0.276$
Observation period: 10 days, water temperature threshold 20 °C			
Legionella	log _3	0.543	$y_{log10\_Lspp} = 0.1255x_{Temp\_3} - 0.596$
HPC 22 °C	**unchanged _2**	**0.577**	$\mathbf{y_{HPC22} = 3{,}612x_{Temp\_2} - 48{,}151}$
HPC 36 °C	**log _1**	**0.544**	$\mathbf{y_{log10\_HPC36} = 0.1405x_{Temp\_1} + 0.1385}$

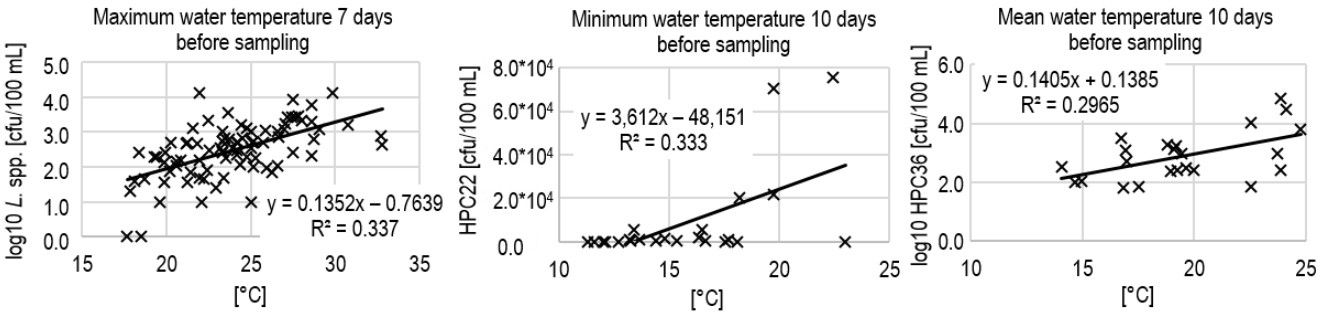


Figure 24: CT2 - Regression lines of microbiological concentrations and modified data derived from the water temperature data.

For HPC at 22 °C, the highest correlations were obtained for the combinations of the unchanged HPC 22 °C data set with the data sets of the minimum water temperatures within the period before sampling. The maximum correlation of r = 0.577 was obtained for the data set ten days before sampling. The regression line is shown in the mid chart of Figure 24. The slope is positive indicating that an increased minimum water temperature is associated with increased HPC at 22 °C. Most data points are located in the lower area of the ordinate. Four data points extend over the lower middle and upper ordinate range and strongly influence the course of the regression line. Due to the y-axis intercept in the five-digit negative range, the HPC is only positive for minimum water temperatures higher than 13.3 °C. With a plausible high minimum water temperature of 25 °C the maximum calculable HPC amounts to $4.2\,10^4$ cfu/mL.

For HPC at 36 °C, the highest correlations were obtained for the observation period of five days before sampling for the combination of log-transformed HPC 36 °C-values and the minimum water temperature. For the observation periods of seven and ten days the highest correlations were received for the log-transformed HPC 36 °C-values and the mean water temperature before sampling. The maximum correlation of $r = 0.544$ was obtained for the data set ten days before sampling. The regression line is shown in the right chart in Figure 24. The slope is positive indicating that an increasing mean water temperature was associated with increased HPC at 36 °C. The point cloud flanked the regression line fairly evenly. With a for Cooling Tower 2 plausible very high average water temperature of 30 °C, a maximum HPC at 36 °C of $2.3 * 10^4$ cfu/mL is calculable.

3.2.1.3 Cooling Tower 3

In Cooling Tower 3, 80 of 84 water samples from 2012 to 2018 contained legionellae. 43 samples showed concentrations between 10^2 and 10^3 cfu/100 mL (see Figure 35). In 29 samples, lower concentrations of up to 10^2 cfu/100 mL were found. In eight samples, concentrations between 10^3 and 10^4 cfu/100 mL were detected. No seasonal fluctuations were deduced from the course of the *Legionella* spp. concentrations over the observation period shown in Figure 25. The concentration seemed to fluctuate independently of the season. Thus, in the begin of the winter months 2012/13 increasing and then decreasing legionellae concentrations were recorded, in the winter months 2013/14 constantly high concentrations were observed. In the winter months 2014/15, however, there was a significant drop in concentration. Even in summer, the course of the legionellae concentration over the observation period was not uniform. After summer 2012, when the concentration was regarded as stable, a steady increase of the *Legionella* spp. concentrations was visible in summer 2013. Summer 2014 showed no fluctuations, while in late summer 2015 an increase in the *Legionella* spp. concentration was noted. In the summer months of 2016, the concentration was initially very low and increased in late summer. A less drastic difference in the concentration curve was visible in summer 2017. In summer 2018, the legionellae concentration fluctuated very strongly.

In the 80 *Legionella* containing samples, 50 times *L. pneumophila* serogroup 1 and 51 times *L. pneumophila* of the serogroup range 2 - 14 were identified. In six samples, *Legionella species* strains that agglutinated in the Latex test and in four samples *Legionella species* strains that did not show a positive reaction in the Latex test were found (see Figure 26). In 32 samples, two or more *Legionella* strains were recorded at the same time.

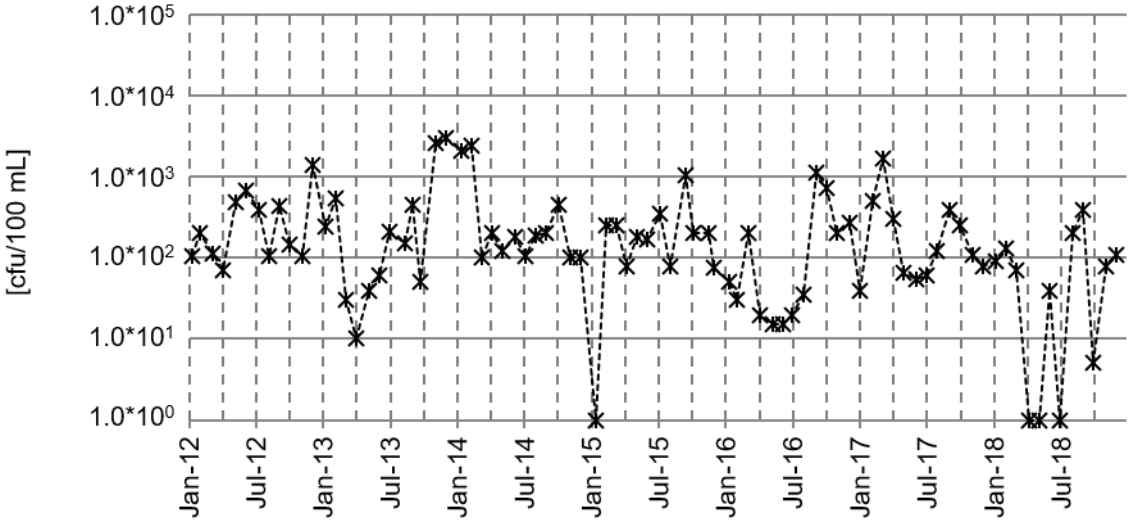


Figure 25: CT3 - *Legionella* spp. concentrations from 2012 to 2018.

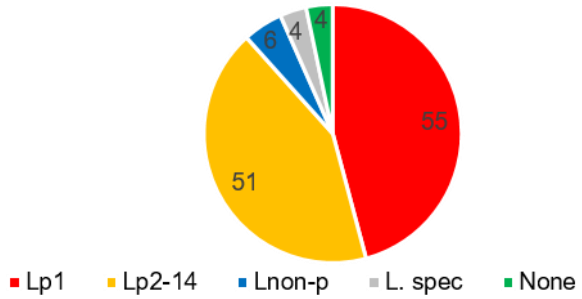


Figure 26: CT3 – Distribution of *Legionella* strains in 84 samples from 2012 to 2018.

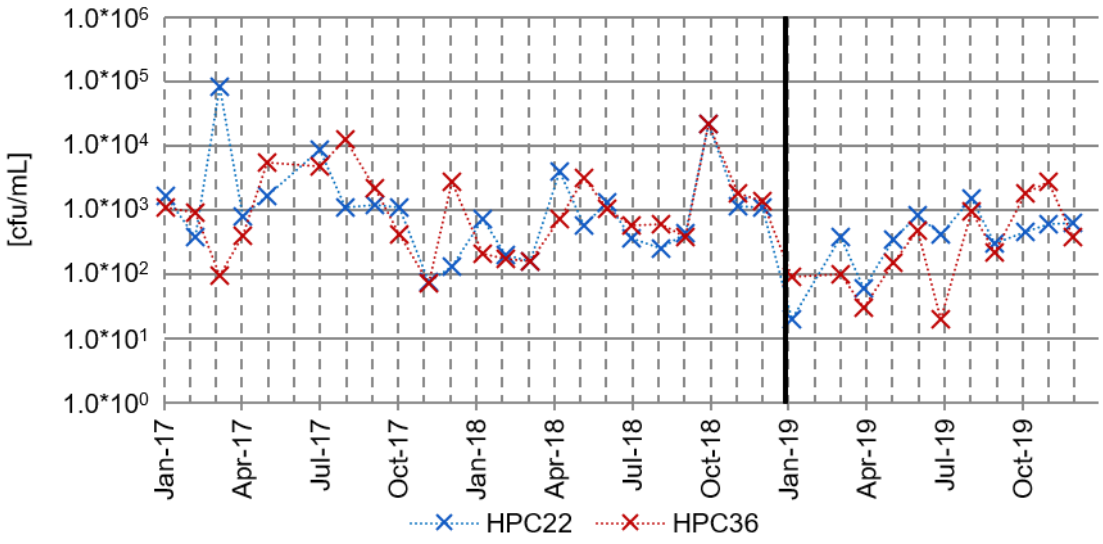


Figure 27: CT3 - HPC concentrations from 2017 to 2019.

The curves of the HPC for the years 2017 to 2019 shown in Figure 27 are largely very similar. Considering all 34 investigations, 22 results were in the same range when using the internal laboratory rule to consider microbiological results of water samples as equivalent differing less than a half log level. In five samples HPC were higher at 22 °C, in seven samples at 36 °C. The HPC data sets included 34 data for the regression analyses with the redox potential data and 22 for the regression analyses with the water temperature data. In spring 2017, an increase of the HPC was observed. In particular, a peak of the HPC at 22 °C in March 2017 was noted. A difference of three log steps was observed between the HPC 22 °C- and the 36 °C-values. In autumn 2017, the HPC decreased, but rose again in December. In spring 2018, the HPC did not seem to fluctuate. In early summer, HPC at both incubation temperatures increased, decreased during the summer months and then remained stable until September. In October, both concentrations rose by more than one log level, and then fell steadily over two to three log levels until January 2019. The year 2019 was characterized by a steady increase in HPC.

No special dosing with peracetic acid was carried out in Cooling Tower 3 during the observation period.

The box plots in Figure 28 show the unchanged microbiological data sets and the process parameter data sets. Due to the fact that over a long period in November 2018 no water temperature data were recorded, two *Legionella* spp. data sets exist including 84 legionellae data for the regression analyses with the redox potential data and 83 for the analyses with the water temperature data. The determined *Legionella* concentrations ranged from below the detection limit ("one") to 3,080 cfu/100 mL for both data sets. 50 % of the values varied between 61 and 294 cfu/100 mL for the data set correlated with the redox potential data. For the data set correlated with the water temperature data, 50 % of the values varied between 60 and 300 cfu/100 mL. As outliers, ten values each above 643 | 660 cfu/100 mL were determined.

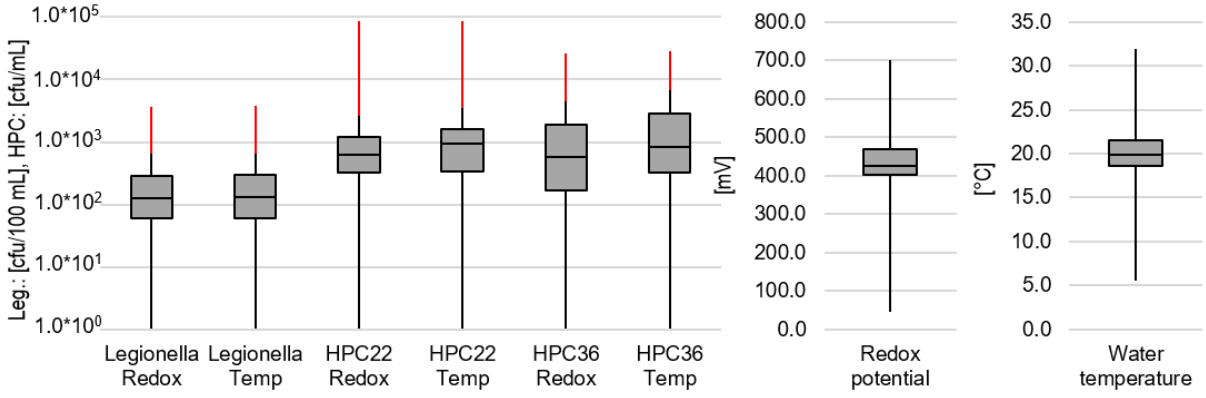


Figure 28: CT3 - Boxplots of the different data sets.
Unchanged microbiological data ("one" corresponds to a result below the detection limit), process parameter data from 2012 to 2018 (water temperature data) and 2012 to 2019 (redox potential data).

The HPC 22 °C | 36 °C data sets used for the regression analyses with the redox potential data ranged from below the detection limit ("one") to 83,754 | 21,600 cfu/mL. The medians were 615 | 575 cfu/mL. Of the HPC results 50 % ranged from 330 to 1,233 | from 168 to 1,908 cfu/mL. Each four values higher than 2,586 | 4,517 cfu/mL were determined as outliers.

The HPC 22 °C | 36 °C data sets used for the regression analyses with the water temperature data ranged from below the detection limit to 83,754 | 21,600 cfu/mL. The medians were 930 | 825 cfu/mL. The lower and upper quartile amounted to 333 and 1,620 | 330 to 1,908 cfu/mL. For HPC at 22 °C, four values were higher than 4,518 cfu/mL. For HPC at 36 °C, two values were higher than 6,693 cfu/mL. These results were determined as outliers.

The number of outliers of the microbiological data sets is listed in Table 25. Due to the removal of implausible data, no outliers existed for the process parameter data sets. The range of the redox potential data (2012 to 2019) extended from 45 to 700 mV. The median amounted to 427 mV. The lower quartile was 402 mV and the upper one was 468 mV. The value range of the water temperature data (2012 to 2018) extended from 5.6 to 31.9 °C. The median amounted to 19.9 °C, the lower quartile to 18.6 °C and the upper one to 21.5 °C.

Table 25: CT3 – Number of outliers of the microbiological data.

Dataset	LEG	log LEG	HPC22	log HPC22	HPC36	log HPC36
Redox potential	2012-2018		2017-2019		2017-2019	
	10	5	4	3	4	0
Water temperature	2012-2018		2017-2018		2017-2018	
	10	5	4	2	2	0

The regression analyses of the microbiological data from Cooling Tower 3 with the modified redox potential data showed Pearson correlations higher than 0.5 for HPC at both incubation temperatures indicating a large strength of association (see Table 26). For both incubation temperatures, the data sets of the time below the redox potential threshold value of 400 mV within ten days before sampling provided the highest correlations. For HPC at 22 °C the log-transformed values, for HPC at 36 °C the non-transformed counts were applied.

The regression line of the HPC 22 °C data combined with data set of time below the redox potential threshold value is shown in the left chart of Figure 29. The regression line of the HPC 36 °C data combined with the time below the redox potential threshold value is shown in the right chart. The regression lines have positive slopes indicating that an increasing time below the threshold value is associated with increased HPC. In both charts, a large part of the data points flanks the left part of the regression line. In the 22 °C-HPC chart, the remaining area of the regression line is moderately evenly surrounded. In the 36 °C-HPC chart, seven data points extend over a quite wide range on the abscissa, but only over a small range on the ordinate. One single data point in the upper right corner strongly influenced the course of the regression

line. Theoretically, the time below the threshold value can reach a maximum value of 240 hours. Consequently, the maximum calculable HPC 22 °C-value amounts to $4.6 * 10^4$ cfu/mL and $1.3 * 10^4$ cfu/mL for HPC at 36 °C.

Table 26: CT3 - Results of the regression analyses of the microbiological and redox potential data.

Parameter	Datasets	Correlation r (Pearson)	Assessment / Formula
Observation period: 5 days, redox potential threshold: 400 mV			
Legionella	unchanged _5	-0.289	Correlation not significant
HPC 22 °C	log _6	0.450	$y_{log10\_HPC22} = 0.0146x_{Redox\_6} + 2.6309$
HPC 36 °C	unchanged _6	0.497	$y_{HPC36} = 98x_{Redox\_6} + 666$
Observation period: 7 days, redox potential threshold: 400 mV			
Legionella	unchanged _5	-0.290	Correlation not significant
HPC 22 °C	log _6	0.534	$y_{log10\_HPC22} = 0.0119x_{Redox\_6} + 2.5939$
HPC 36 °C	unchanged _6	0.555	$y_{HPC36} = 75x_{Redox\_6} + 513$
Observation period: 10 days, redox potential threshold: 400 mV			
Legionella	unchanged _5	-0.267	Correlation not significant
HPC 22 °C	**log _6**	**0.591**	$\mathbf{y_{log10\_HPC22} = 0{,}0087x_{Redox\_6} + 2.5761}$
HPC 36 °C	**unchanged _6**	**0.571**	$\mathbf{y_{HPC36} = 51x_{Redox\_6} + 513}$

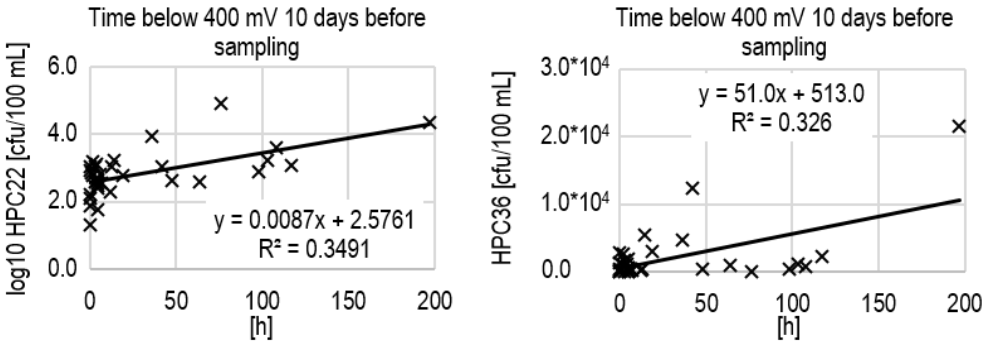


Figure 29: CT3 - Regression lines of microbiological concentrations and modified data derived from the redox potential data.

No regression analyses of the combination of *Legionella* spp. and a modified data set of the redox potentials provided a Pearson correlation higher than 0.3. Respectively no regression line was created.

The regression analyses of the microbiological data sets from Cooling Tower 3 with the modified data of the water temperatures did not provide a Pearson correlation higher than 0.3 (see Table 27). Due to the too high error probability of the correlation of the data sets no regression lines were plotted.

Table 27: CT3 - Results of the regression analyses of the microbiological and water temperature data.

Parameter	Datasets	Correlation r (Pearson)	Assessment / Formula
Observation period: 5 days, water temperature threshold 20 °C			
Legionella	unchanged _2	-0.252	Correlation not significant
HPC 22 °C	unchanged _4	-0.271	Correlation not significant
HPC 36 °C	unchanged _2	0.144	Correlation not significant
Observation period: 7 days, water temperature threshold 20 °C			
Legionella	unchanged _2	-0.213	Correlation not significant
HPC 22 °C	unchanged _4	-0.252	Correlation not significant
HPC 36 °C	unchanged _2	0.124	Correlation not significant
Observation period: 10 days, water temperature threshold 20 °C			
Legionella	unchanged _4	-0.156	Correlation not significant
HPC 22 °C	unchanged _4	-0.250	Correlation not significant
HPC 36 °C	unchanged _2	0.130	Correlation not significant

3.2.1.4 Cooling Tower 4

The *Legionella* concentration in Cooling Tower 4 was subject to strong fluctuations as shown in Figure 30. The concentrations tended to be higher in summer. In the years 2012 to 2014, the *Legionella* spp. concentrations are on average one log level lower than in the years 2015 to 2018. As shown in Figure 30, no legionellae were found in twelve of the 84 samples. In 25 samples, concentrations up to 100 cfu/100 mL were obtained. In 26 samples, concentrations between 10^2 and 10^3 cfu/100 mL and in 19 samples concentrations between 10^3 and 10^4 cfu/100 mL were detected. Two results exceeded 10,000 cfu/100 mL. In the 72 legionellae containing samples, 20 times *L. pneumophila* serogroup 1 and 62 times *L. pneumophila* of the serogroup range 2 - 14 were identified. In two samples, *Legionella species* strains were detected that did not show a positive reaction in the Latex test (see Figure 31). In twelve samples, two or more *Legionella* strains were detected at the same time. During the observation period, six special dosages of peracetic acid were made. The dosages in September 2015 and October 2017 seem to have been carried out due to high *Legionella* spp. concentrations. They have led to a reduction of the *Legionella* concentration. The special dosage in March 2015 was probably carried out due to high algae contamination. It is not clear whether the peracetic acid doses in October 2016 and in June and August 2018 were carried out due to high *Legionella* spp. concentrations. After the special dosing in October 2016, the concentration in the cooling water increased significantly to over 10^4 cfu/100 mL, but decreased to 5 cfu/100 mL in the following months. Even after the dosage in June 2018, the concentration was initially reduced by three log steps, but increased again by almost the same factor in the following month. After

the dosage in August 2018, a steady reduction of the legionellae concentration by one log level was observed.

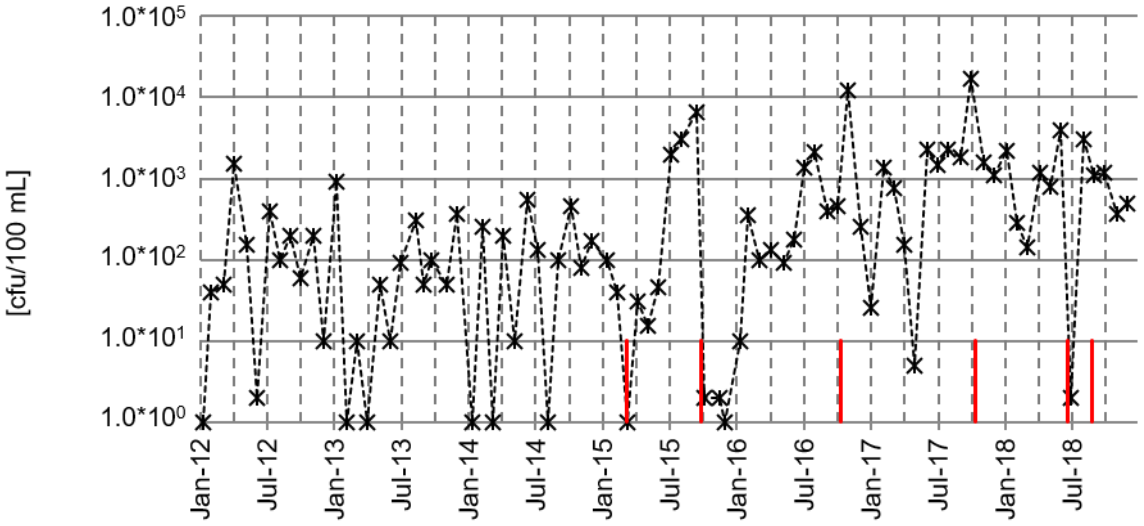


Figure 30: CT4 - *Legionella* spp. concentrations from 2012 to 2018.
Red lines: special peracetic acid biocide dosages.

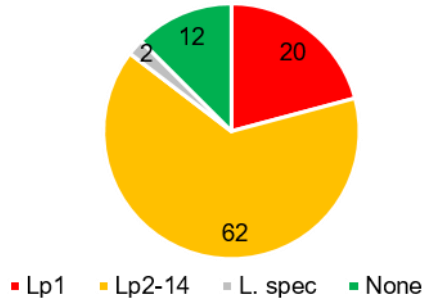


Figure 31: CT4 – Distribution of *Legionella* strains in 84 samples from 2012 to 2018.

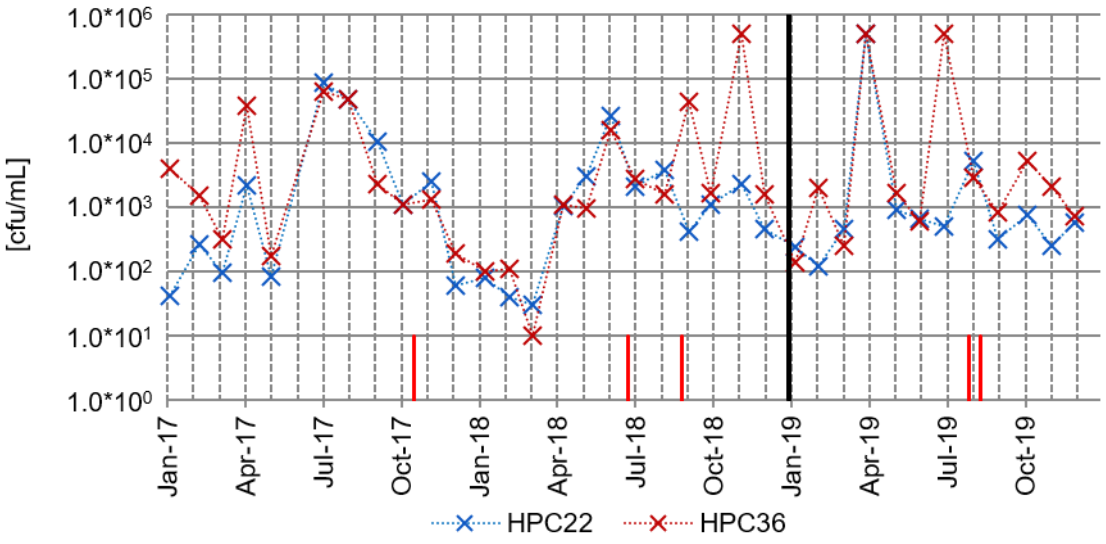


Figure 32: CT4 - HPC concentrations from 2017 to 2019.
Red lines: special peracetic acid biocide dosages.

The HPC curves of both incubation temperatures shown in Figure 32 were similar for many measurements. However, in some cases the results for HPC at 36 °C were noticeably higher. Considering all 35 investigations, 22 results were in the same range when using the internal laboratory rule to consider microbiological results of water samples as equivalent if they differ less than a half log level. One sample had higher HPC at 22 °C, twelve at 36 °C. In winter 2017/18, a significant decrease of three log steps until spring 2018 was noted for both curves. From this point on, both curves rose again by almost three log steps until June. The HPC at 22 °C curve fell until February 2019, and then increased rapidly within two months. After this rapid rise, the HPC at 22 °C decreased until the end of the recordings. The course of the HPC at 36 °C curve deviated noticeably from the HPC 22 °C-curve after the June 2018 measurement. The HPC 36 °C curve fluctuated strongly showing three outstanding peaks in November 2018, April and June 2019, after which the HPC 36 °C dropped again. It is possible that the special dosage was carried out at the end of July due to the very high HPC at 36 °C incubation temperature. After the dosages, the HPC at 36 °C decreased steadily. The HPC data sets included 35 data for the regression analyses with the redox potential data and 23 for the regression analyses with the water temperature data.

The box plots in Figure 33 show the unchanged microbiological datasets and the process parameter data sets. The determined legionellae concentrations ranged from below the detection limit (“one”) to 17,000 cfu/100 mL. The lower quartile amounted to 33 cfu/100 mL and the upper one to 1,050 cfu/100 mL with the median at 163 cfu/100 mL. Six values above 2,577 cfu/100 mL were determined as outliers.

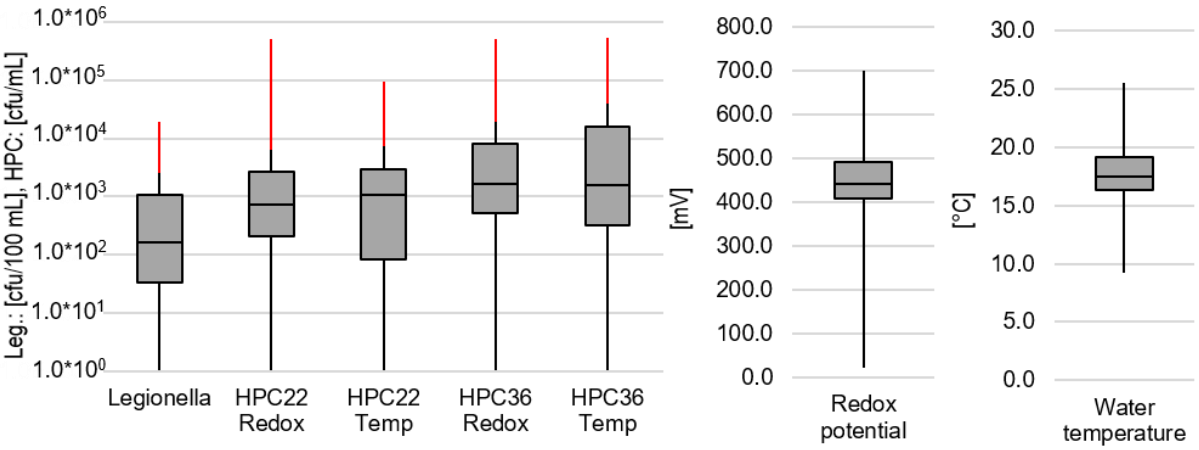


Figure 33: CT4 - Boxplots of the different data sets.
Unchanged microbiological data (“one” corresponds to a result below the detection limit), process parameter data from 2012 to 2018 (water temperature data) and 2012 to 2019 (redox potential data).

The HPC 22 °C | 36 °C data sets used for the regression analyses with the redox potential data ranged from below the detection limit to 500,000 cfu/mL each (exceedance of the upper quantification limit; the exact value was not determinable). The medians amounted to

715|1,645 cfu/mL. Of the values 50 % varied between 210 and 2,625|529 and 8,100 cfu/mL. For HPC at 22 °C, five values were higher than 6,248 cfu/mL. For HPC at 36 °C, eight values were higher than 19,457 cfu/mL. These results were determined as outliers.

The HPC 22 °C|36 °C data sets used for the regression analyses with the water temperature data range from "one" (< DL) to 86,400|500,000 cfu/mL (> UQL, see above). The medians were 1,080|1,590 cfu/mL. The lower and upper quartiles ranged of the HPC ranged from 82 to 3,000|315 to 16,200 cfu/mL. Four values higher than 7,377|40,027 cfu/mL were each determined as outliers.

The number of outliers of the microbiological data sets is listed in Table 28. Due to the removal of implausible data, no outliers existed for the process parameter data sets. The value range of the redox potential data (2012 to 2019) extended from 20 to 700 mV. The median amounted to 441 mV. The range between the lower and upper quartile contained results from 407 to 491 mV. The range of the water temperature data (2012 to 2018) extended from 9.2 to 25.5 °C. The median amounted to 17.5 °C, the lower quartile to 16.4 °C and the upper quartile to 19.1 °C.

Table 28: CT4 – Number of outliers of the microbiological data.

Dataset	LEG	log LEG	HPC22	log HPC22	HPC36	log HPC36
Redox potential	2012-2018		2017-2019		2017-2019	
	6	0	5	1	7	3
Water temperature	2012-2018		2017-2018		2017-2018	
	6	0	4	0	4	0

The regression analyses of the microbiological data sets from Cooling Tower 4 with the modified data of the redox potential data did not provide a Pearson correlation higher than 0.3 (see Table 29). Due to the too high error probability of the correlation of the data sets, no regression lines were plotted.

Table 29: CT4 - Results of the regression analyses of the microbiological and redox potential data.

Parameter	Datasets	Correlation r (Pearson)	Assessment / Formula
Observation period: 5 days, redox potential threshold: 400 mV			
Legionella	log _2	-0.210	Correlation not significant
HPC 22 °C	log _2	0.210	Correlation not significant
HPC 36 °C	unchanged _5	0.282	Correlation not significant
Observation period: 7 days, redox potential threshold: 400 mV			
Legionella	log _2	-0.224	Correlation not significant
HPC 22 °C	log _2	0.289	Correlation not significant
HPC 36 °C	unchanged _5	0.237	Correlation not significant
Observation period: 10 days, redox potential threshold: 400 mV			
Legionella	log _6	-0.255	Correlation not significant
HPC 22 °C	log _2	0.289	Correlation not significant
HPC 36 °C	unchanged _5	0.219	Correlation not significant

The regression analyses of all log-transformed microbiological data in combination with the modified water temperature data sets provided Pearson correlation values higher than 0.4 (see Table 30).

Table 30: CT4 - Results of the regression analyses of the microbiological and water temperature data.

Parameter	Datasets	Correlation r (Pearson)	Assessment / Formula
Observation period: 5 days, water temperature threshold 20 °C			
Legionella	unchanged _3	0.384	$y_{Lspp} = 238x_{Temp\_3} - 5{,}500$
HPC 22 °C	**log _4**	**0.626**	$\mathbf{y_{log10\_HPC22} = 0.0123x_{Temp\_4} + 2.1625}$
HPC 36 °C	log _4	0.351	$y_{log10\_HPC36} = 0.0074x_{Temp\_4} + 2.8366$
Observation period: 7 days, water temperature threshold 20 °C			
Legionella	**unchanged _3**	**0.388**	$\mathbf{y_{Lspp} = 264x_{Temp\_3} - 6{,}080}$
HPC 22 °C	log _4	0.620	$y_{log10\_HPC22} = 0.0087x_{Temp\_4} + 2.1617$
HPC 36 °C	log _4	0.351	$y_{log10\_HPC36} = 0.0053x_{Temp\_4} + 2.8314$
Observation period: 10 days, water temperature threshold 20 °C			
Legionella	log _4	0.368	$y_{log10\_Lspp} = 0.0042x_{Temp\_4} + 1.6429$
HPC 22 °C	log _4	0.607	$y_{log10\_HPC22} = 0.0063x_{Temp\_4} + 2.1137$
HPC 36 °C	**log _4**	**0.356**	$\mathbf{y_{log10\_HPC36} = 0.004x_{Temp\_4} + 2.7837}$

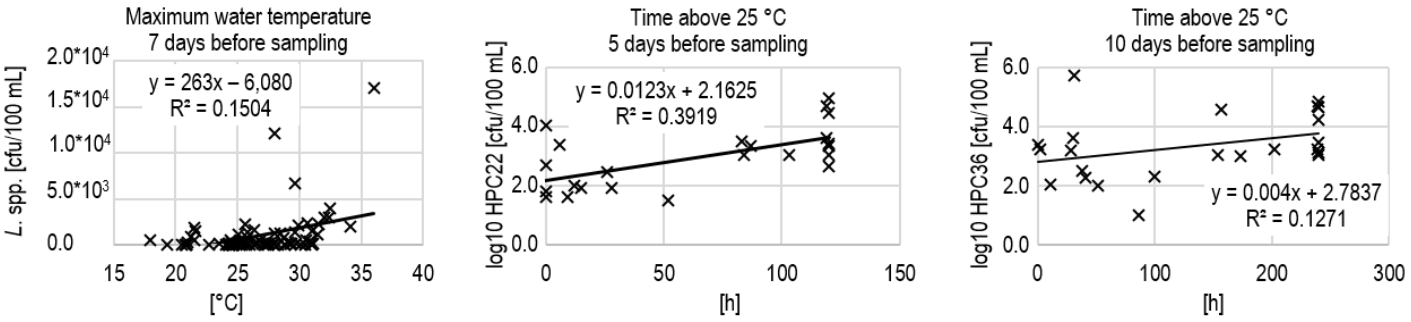


Figure 34: CT4 - Regression lines of microbiological concentrations and modified data derived from the water temperature potential data

For *Legionella* spp. the maximum correlation (r = 0.388) was obtained for the combination of the unchanged *Legionella* spp. data and the data set of the maximum water temperature within seven days before sampling. The corresponding regression line is shown in the left chart of Figure 34. The regression line has a positive slope indicating that an increased maximum water temperature is associated with increased legionellae concentrations. For a plausible very high maximum water temperature of 40 °C within seven days before sampling a maximum *Legionella* concentration of $4.4 * 10^4$ cfu/100 mL is computable by the regression line. Because of the negative y-axis intercept *Legionella* concentrations in the positive range are obtained for maximum water temperatures above 23.1 °C within seven days before sampling.

The highest correlations of the regression analyses of HPC were obtained for both incubation temperatures with the log-transformed HPC data sets in combination with the data sets of the time above the water temperature threshold value of 25 °C. For HPC at 22 °C, the five days observation period showed the highest correlation (r = 0.626). For HPC at 36 °C, the ten days

observation period resulted in the highest correlation (r = 0.356). The corresponding regression line of HPC at 22 °C is shown in the mid chart, the one of HPC at 36 °C in the right chart of Figure 34. Both regression lines have a positive slope indicating that an increased time above the water temperature threshold is associated with increased HPC. Both regression lines are characterized by many data points on the right and left ends of the regression lines. Only a few data points were located in the middle areas of the regression lines. The HPC at 22 °C data points of the mid area of the abscissa are closer to the regression line than those of HPC at 36 °C.

For the five days observation period, the time above the water temperature threshold may receive a maximum value of 120 hours. Respectively, a maximum HPC at 22 °C of $4.4 * 10^3$ cfu/mL is computable by the regression line.

For the ten days observation period, the time above the water temperature threshold value may receive a maximum value of 240 hours. Respectively, a maximum HPC at 36 °C of $5.5 * 10^3$ cfu/mL is computable by the regression line.

3.2.1.5 Distribution of *Legionella* concentrations according to 42nd BImSchV in the four cooling towers

With the introduction of the VDI Code of Practice (159) and the 42nd BImSchV (1) the *Legionella* contamination in cooling towers has gained enormous importance. The distribution of the *Legionella* concentrations in the four investigated cooling towers according to the test and action values of the 42nd BImSchV is described in this subsection.

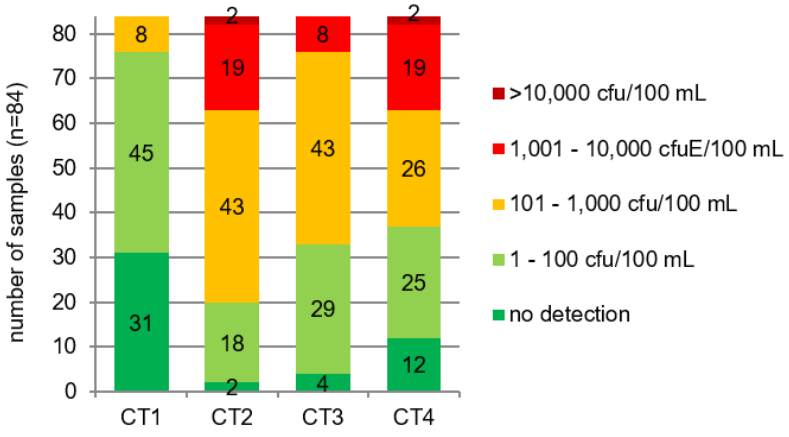


Figure 35: Distribution of the *Legionella* concentrations in the four cooling towers from 2012 to 2018 divided according to the test and action values of the 42nd BImSchV.

As illustrated in Figure 35, in Cooling Tower 1 most commonly no or low concentrations below 100 cfu/100 mL were detected. Concentrations above 1,000 cfu/100 mL were not detected in the 7-year observation period. In Cooling Tower 4, the second most frequently no *Legionella*

or low concentrations below the first test value of the 42nd BImSchV results were obtained. In this cooling tower also exceedances of the test value 2 (> 1,000 cfu/100 mL) and the action value (> 10,000 cfu/100 mL) were recorded. Cooling Tower 3 showed the third most frequently results below test value 1. Test value 2 was exceeded eight times, but the action value was not exceeded. Cooling Tower 2 showed the fewest results below test value 1. As in Cooling Tower 3, half of the Cooling Tower 2 results exceeded test value 1. With the same number as in Cooling Tower 4, the test value 2 and the action value were exceeded in Cooling Tower 2.

In sections 3.2.1.1 to 3.2.1.4, the distributions of *Legionella* strains were shown in the circle diagrams. In addition, Table 31 shows how the number of *Legionella* detections was distributed among each of the 84 samples. In Cooling Towers 1 and 2, detections exclusively of *L. pneumophila* strains from serogroups range 2 to 14 were recorded most frequently in each of the 84 samples. The sole occurrence of *L. pneumophila* serogroup 1 was observed second most frequently. In Cooling Tower 3, single contaminations of *L. pneumophila* serogroup 1 strains were found in most samples. Co-contaminations of *L. pneumophila* serogroup 1 and from the range of serogroups 2 to 14 were recorded second most frequently. Only *L. pneumophila* strains from the range of serogroups 2 to 14 were recorded third most frequently. In Cooling Tower 4, strains exclusively of *L. pneumophila* strains from the range of serogroups 2 to 14 were observed in most samples. Co-contaminations of *L. pneumophila* serogroup 1 and from the range of serogroups 2 to 14 were detected second most frequently. *L. pneumophila* serogroup 1 occured third most frequently. Only in Cooling Tower 4, the sole occurrence of an *Legionella species* strain was observed in one sample. Co-contaminations of *Legionella* species strains and *L. pneumophila* serogroup 1 or 2 to 14 strains were observed in each one sample of Cooling Towers 1, 2 and 4 and in four samples of Cooling Tower 3. Detections of only *L*. non-*pneumophila* strains or co-contaminations of *L*. non-*pneumophila* and *Legionella species* strains were not recorded in any sample. Co-contaminations of *L. non-pneumophila* strains and *L. pneumophila* serogroup 1 or 2 to 14 strains were recorded in six samples of Cooling Tower 3.

Table 31: *Legionella species* detections in each 84 samples from the four cooling towers.

	CT1	CT2	CT3	CT4
Lp SG 1	1	27	26	9
Lp SG 2-14	46	38	21	51
L. sp.	0	0	0	1
Lp SG 1, 2-14	5	16	23	11
Lp SG 1, *L.* sp.	0	1	2	0
Lp SG 2-14, *L.* sp.	1	0	2	1
Lp SG 1, *L.* non-*pneumophila*	0	0	1	0
Lp SG 2-14, *L.* non-*pneumophila*	0	0	2	0
Lp SG 1, Lp SG 2-14, *L.* non-*pneumophila*	0	0	3	0
No *Legionella* detected	31	2	4	11

3.2.2 Seasonal dynamics in the four cooling towers

Since most legionellosis outbreaks occurred in the warmer months (164), the following section investigated whether and which seasonal microbiological dynamics existed in the four cooling towers. Planktonic *Legionella* concentrations from 2012 to 2018 and HPC data from 2017 to 2019 were included. The left charts of Figure 36 show the monthly *Legionella* concentrations over the seven-year observation period by boxplots. The boxplots of the right charts contain the seven-year *Legionella* concentrations of the "cold" months, October to March, and the "warm" months, April to September. Also in Figure 37, for each incubation temperature, the left charts show the monthly recorded HPC over the three-year observation period. Minimum, median and maximum have been plotted. Sometimes only two values were recorded within the three years, for example when agar plates could not be evaluated. For each incubation temperature, the right charts include the HPC of the “cold” and “warm” months.

All cooling towers show seasonal variability for both *Legionella* and HPC. In Cooling Towers 1 and 3 seasonal fluctuations are less prominent than in Cooling Towers 2 and 4. The highest average *Legionella* concentrations were recorded between August and October, except for Cooling Tower 1. Regarding the monthly boxplots of Cooling Towers 2 and 4, an increase in *Legionella* between March and August/September and a decrease between September/October and March was observed. For both cooling towers, the interquartile ranges of the boxplots of the warm months are above those of the cold months. In Cooling Towers 1 and 3, wavelike *Legionella* concentrations were noticed throughout the year. In Cooling Tower 1, only moderate fluctuations occur, decreasing between January and June, showing a characteristic gap in July, increasing towards August and dropping slightly until February. Peaks occur in January, May, September and December. The boxplots of the cold and warm months are almost congruent. In Cooling Tower 3, the concentrations oscillate over the year with peaks in February, July and November. The boxplot of the cold months shows a higher lower quartile and maximum. Median and upper quartile are almost the same for both boxplots.

In each cooling tower, the seasonal fluctuations of HPC are very similar for both incubation temperatures. As for *Legionella*, the HPC fluctuations are more obvious in Cooling Towers 2 and 4 than in Cooling Towers 1 and 3. The boxplots of the cold and warm months also show this. HPC tends to increase in the warm months and the highest HPC is recorded in July and August. Peaks in cold months are noticeable in Cooling Tower 4.

To confirm the subjective impression, the Mann-Whitney-U-test (two-tailed hypothesis, significance level at 5 %) was used to test whether the data sets differ for the warm and cold months even if data sets were not large. Significant differences were recorded in Cooling Tower 2 for all parameters and for Cooling Tower 4 for HPC.

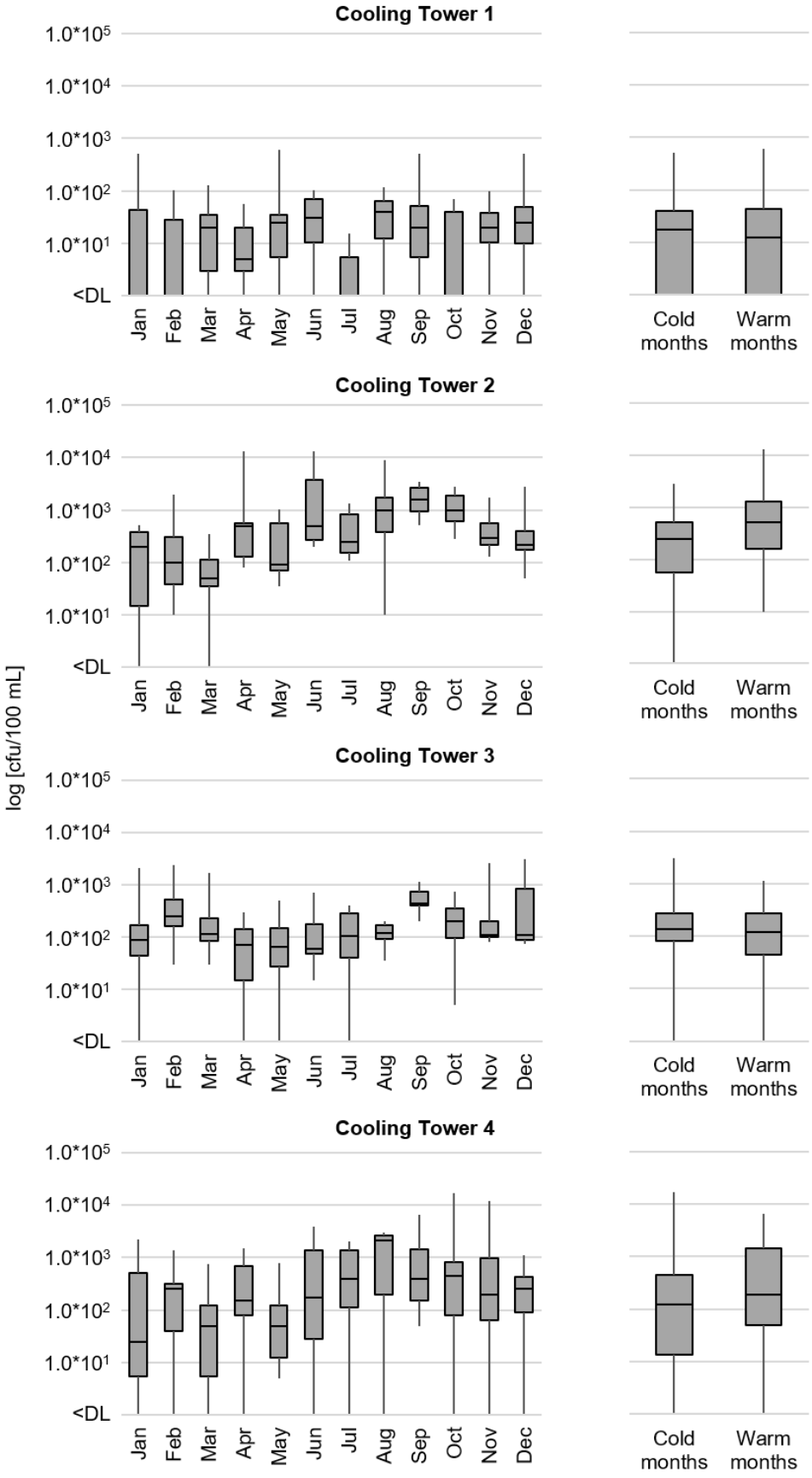


Figure 36: Seasonal dynamics of *Legionella*.
Left charts show box plots of monthly detected *Legionella* concentrations between 2012 and 2018. Right charts summarise the concentrations for cold (October to March) and warm months (April to September).

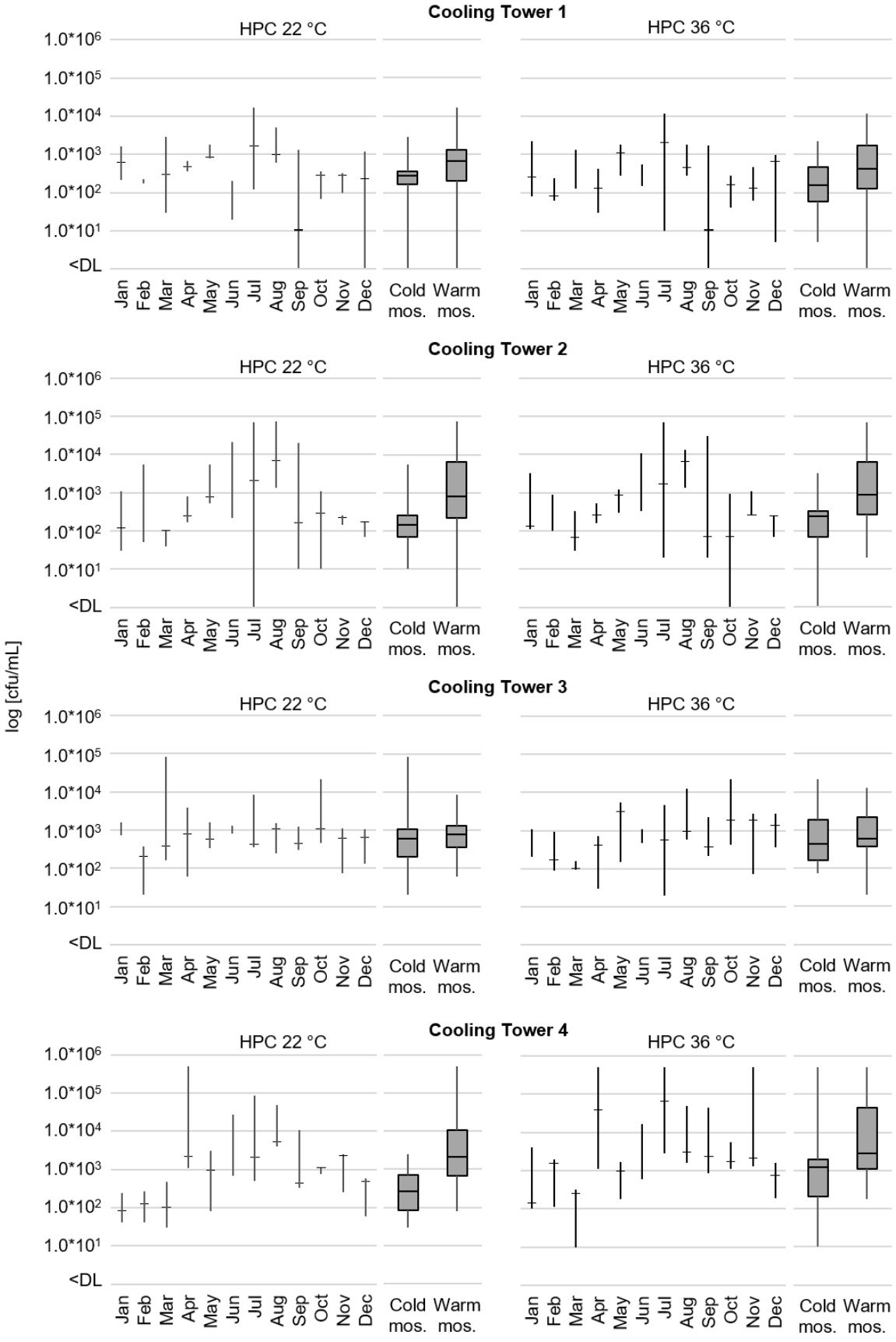


Figure 37: Seasonal dynamics of HPC.
For both incubation temperatures, the left charts show monthly detected HPC between 2017 and 2019. Right charts summarise the concentrations for cold (October to March) and warm months (April to September).

3.2.3 Comparison of heterotrophic plate counts at the incubation temperatures of 22 and 36 °C detected with the ISO method

The detection of heterotrophic plate counts according to 42nd BImSchV is performed to estimate the microbial status in the entire cooling system and to detect an increased risk of biofilm formation at an early stage. The ISO method (73) has to be applied according to 42nd BImSchV. This method was originally designed for the detection of HPC in drinking water samples (172). Industrial water samples are often highly contaminated by microorganisms that grow under the given nutrient and incubation conditions specified by the method. Tiny colonies, dirt particles in the sample and colonies with swarm behaviour complicate the counting and consequently influence the measurement uncertainty as well as the comparability of results. Smaller volumes and / or dilution series are necessary to obtain reliable results. This means a high workload for the laboratories. The ISO method offers a lot of flexibility in sample processing and reading the plates. This leads to a large dispersion of the results (90). So far, there is no generally applicable or binding recommendation how to process industrial samples or samples with a high microbial background. A comparative analysis of the HPC results was carried out to check if it is possible to perform only one incubation temperature to reduce the workload without the risk of loss of information.

The comparison included the HPC results of 2,868 industrial samples from the Laboratory for Technical Hygiene at IHPH. Only results obtained with the ISO method were considered. The ISO method was introduced on November 1st 2018 in the Laboratory for Technical Hygiene. Of the 2,868.samples, 2,745 samples complied with the scope of the 42nd BImSchV. The remaining 123 samples derived from other industrial sites. To get a first overview how the results of the two incubation temperatures were classified, Table 32 was compiled.

Regarding the total data set in Table 32 a, 76.3 % of the samples were quantifiable with both methods and 11.4 % showed results below the quantification limit. Samples that were quantifiable at 36 °C but showed results below the detection limit or above the upper detection limit at 22 °C amounted to 6.0 %. The amount is 5.3 % vice versa. For both incubation temperatures 0.9 % of the samples showed not evaluable results or results above the upper quantification limit.

Regarding the data set containing samples subject to the scope of the 42nd BImSchV in Table 32 b, 75.8 % of the samples were quantifiable with both methods and 11.5 % showed results below the quantification limit. Samples that were quantifiable at 36 °C but showed results below the detection limit or above the upper detection limit at 22 °C amounted to 6.2 %. The amount is 5.3 % vice versa. For both incubation temperatures 5.4 % of the samples showed not evaluable results or results above the upper quantification limit.

Table 32: HPC results of industrial samples from the Laboratory of Technical Hygiene.
DL: detection limit; UQL: upper quantification limit.

	a) Total sample set	**36 °C**			
	n = 2.868	< DL	quantifiable	> UQL	not evaluable
22 °C	< DL	**328**	145	0	3
	quantifiable	91	**2.188**	40	20
	> UQL	0	13	**20**	0
	not evaluable	0	15	0	**5**
	b) 42nd BImSchV	**36 °C**			
	n = 2.745	< DL	quantifiable	> UQL	not evaluable
22 °C	< DL	**316**	141	0	3
	quantifiable	90	**2.083**	39	20
	> UQL	0	13	**20**	0
	not evaluable	0	15	0	**5**
	c) Other industrial sites	**36 °C**			
	n = 123	< DL	quantifiable	> UQL	not evaluable
22 °C	< DL	**12**	4	0	0
	quantifiable	1	**105**	1	0
	> UQL	0	0	**0**	0
	not evaluable	0	0	0	**0**

The data set of the other industrial sites showed that 85.4 % of the samples were quantifiable for both incubation temperatures and 11.4 % were negative. The proportion of samples that were only quantifiable at one incubation temperature was negligible at 4.9 %.

It is noticeable that for both the total data set (see Table 32 a) and the 42nd BImSchV data set (see Table 32 b), almost the same number of samples showed a quantifiable result for only one incubation temperature. Samples that were quantifiable at 36 °C but not at 22 °C were predominantly below the detection limit at 22 °C. Among the samples that were only quantifiable at 22 °C, the samples that were below the detection limit at 36 °C also formed the largest proportion, but in comparison, obviously more samples were not evaluable or above the upper quantification limit at 36 °C. In fact, twice as many samples were quantifiable at 22 °C and not evaluable or above the upper quantification level at 36 °C as vice versa. One third more samples were quantifiable at 36 °C and below the detection limit at 22 °C as vice versa.

Table 32 shows the distribution of the samples that provided a quantifiable result at both temperatures. For the data sets "Total" and "42nd BImSchV" 64.5 % of the results were in the same range, considering the classification in Table 33. The incubation temperature at 22 °C provided 15.4 % ("Total") and 15.7 % ("42nd BImSchV") higher results. For 36 °C 20.0 % ("Total") and 19.7 % ("42nd BImSchV") higher results were recorded. For the dataset of other industrial sites, 64.8 % were in the same range. At 22 °C 9.5 % of the results were in a higher range and at 36 °C 25.7 %.

Table 33: Distribution of quantifiable HPC at the incubation temperatures of 22 and 36 °C.

	a) Total sample set n = 2,188	36 °C			
		< 100 [cfu/mL]	101 - 1,000 [cfu/mL]	1,001 - 10,000 [cfu/mL]	> 10,000 [cfu/mL]
22 °C	< 100 [cfu/mL]	**162**	103	18	5
	101 - 1,000 [cfu/mL]	110	**413**	166	38
	1,001 - 10,000 [cfu/mL]	8	85	**353**	108
	> 10,000 [cfu/mL]	4	23	108	**484**
	b) 42nd BImSchV n = 2,083	**36 °C**			
		< 100 [cfu/mL]	101 - 1,000 [cfu/mL]	1,001 - 10,000 [cfu/mL]	> 10,000 [cfu/mL]
22 °C	< 100 [cfu/mL]	**160**	99	17	5
	101 - 1,000 [cfu/mL]	108	**397**	154	33
	1,001 - 10,000 [cfu/mL]	8	84	**329**	103
	> 10,000 [cfu/mL]	4	23	101	**458**
	c) Other industrial sites n = 105	**36 °C**			
		< 100 [cfu/mL]	101 - 1,000 [cfu/mL]	1,001 - 10,000 [cfu/mL]	> 10,000 [cfu/mL]
22 °C	< 100 [cfu/mL]	**2**	4	1	0
	101 - 1,000 [cfu/mL]	2	**16**	12	5
	1,001 - 10,000 [cfu/mL]	0	1	**24**	5
	> 10,000 [cfu/mL]	0	0	7	**26**

Although the internal "0.5 log level-rule" is originally used to compare sample results tested with the same method, it has been applied here to compare the counts for the two incubation temperatures. The aim was to get an impression how the results of the two incubation temperatures differ and whether one temperature gives more often higher results. The corresponding diagrams are shown in Figure 38. The distribution indicates that for the "Total" and "42nd BImSchV" data sets about one third of the result differences within half a log level or for one incubation temperature each higher. The largest proportion of the "Total" and "42nd BImSchV" data sets are results differing less than half a log level. The proportion of results that differed by more than half a log level was recorded slightly more often for counts higher at 36 °C than at 22 °C. For the data set of the other industrial sites, 40.0 % of the results differed less than half a log level. The largest proportion consisted of paired data where the count was higher at 36 °C differing more than half a log level.

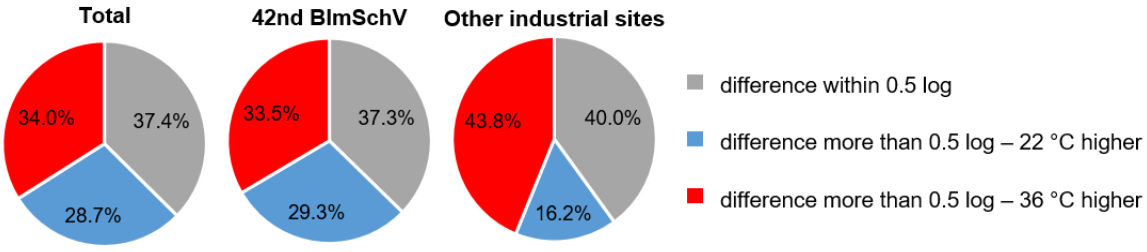


Figure 38: Distribution of quantifiable HPC according to the laboratory intern "0.5 log level-rule"

Statistical analyses were performed for the three data sets to check whether the data sets for incubation temperatures of 22 and 36 °C were significantly different. All paired results (HPC at 22 °C and 36 °C from the same sample) were excluded from the analyses where at least one

result was above the upper quantification limit or not evaluable. First, the data sets were checked for normal distribution. All data sets were not normally distributed with a probability of error less than 5 %. The Wilcoxon signed-rank test showed for all three data sets that the results for 22 and 36 °C were significantly different with a probability of error below 5 %. Furthermore, a relative difference analysis according to ISO 17994 was performed. For the 17994 analysis, paired results were excluded which lay below the detection limit at both incubation temperatures and which exceeded the upper quantification limit or were not evaluable at least at one incubation temperature. The results are listed in Table 34. The locations of the “confidence intervals” are shown in Figure 39.

The rows shown in Table 34 a) to c) and the corresponding “confidence intervals” in Figure 39 represent the paired data that were used for analysis according to ISO 17994 including paired data where one temperature gave a “zero” count. For the 17994 analysis, paired results were excluded which lay below the detection limit at both incubation temperatures and which, at least at one incubation temperature, exceeded the upper quantification limit or were not evaluable. Thus, the paired data, which were below the detection limit at 22 °C but quantifiable at 36 °C, influenced the analysis. This was true for 145 paired data of the total data set and for 141 of the 42nd BImSchV data set (Table 32). In contrast, paired data that were quantifiable at 22 °C but above the quantification limit or not evaluable at 36 °C were not included in the ISO 17994 analysis. This was true for 60 paired data of the total data set and for 59 of the 42nd BImSchV data set (see Table 32). For this reason, an additionally ISO 17994 analysis was carried out including only quantifiable paired data were. These analyses are shown in Table 34 d) and e) and the corresponding “confidence intervals” in Figure 39 d) and e).

Regarding the data sets in Table 34 a) to c) and considering 20 % as a permissible difference limit, the ISO 17994 outcomes indicated that the results of the three data sets differed significantly for the two incubation temperatures. Since the 36 °C counts were subtracted from the 22 °C counts, the negative sign indicated that the results were significantly higher for 36 °C. Due to the generally high dispersion of HPC in cooling tower water samples (90), an extension of the difference limits should be allowed and acceptable. With a difference limit of 30 %, the ISO outcome indicated for the "Total" and "42nd BImSchV" data sets that the statistical difference was indifferent. For the “other industrial sites” data set the difference remained significant.

Table 34: ISO 17994 comparative analyses for HPC at the incubation temperatures of 22 and 36 °C.

[a] Half width of the "confidence interval" around the mean relative difference. [b] Value of the relative difference at the lower "confidence limit". [c] Value of the relative difference at the upper "confidence limit". [d] outcome shown for difference limit of ±20% or ±30%.

Data set	Number of results	Mean relative difference [%]	Standard deviation [%]	W [a] [%]	X_L [b] [%]	X_U [c] [%]	Outcome[d]
a) Total	2,424	-20.9	184.5	7.5	-28.4	-13.4	±20%: at 36°C higher ±30%: indifferent
b) 42nd BImSchV	2,314	-18.4	184.5	7.7	-26.1	-10.7	±20%: at 36°C higher ±30%: indifferent
c) Other industrial sites	110	-74.4	177.0	33.8	-108.1	-40.6	±20%: at 36°C higher
d) Total – only quantifiable results	2,188	-14.1	155.8	6.7	-20.8	-7.4	±20%: at 36°C higher ±30%: indifferent
e) 42nd BImSchV – only quantifiable results	2,083	-11.5	155.1	6.8	-18.3	-4.7	±20%: indifferent

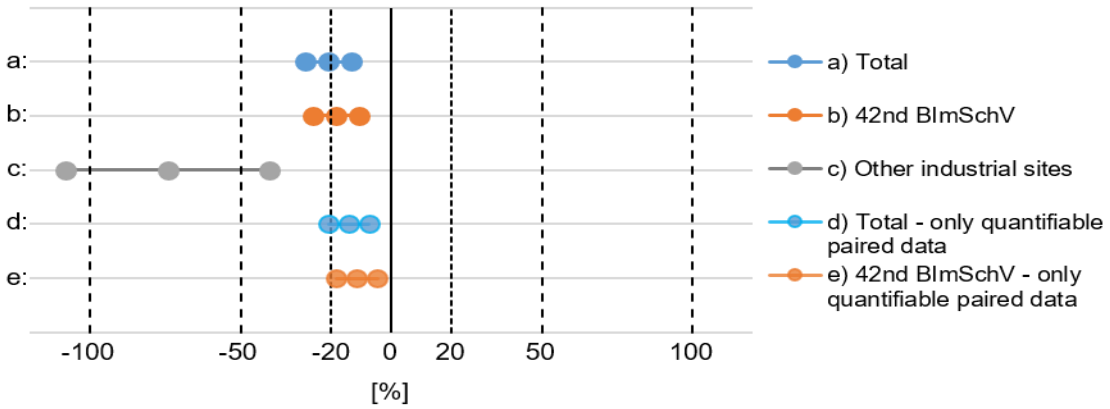


Figure 39: Location of the confidence intervals of relative differences from comparative analyses according to ISO 17994 of paired data from HPC at 22 and 36 °C.

Regarding the data sets d) and e), which included only quantifiable results for both incubation temperatures and considering a permissible difference limit of ±20 %, the ISO 17994 outcome remained significantly higher for the 36 °C results of the "Total" data set. The outcome was indifferent for the "42nd BImSchV" data set. Adjusting the difference limit to ±30 % (even only ±21 %) would result in an indifferent outcome for the "Total" data set.

3.3 Biofilm formation in four different cooling towers

In this chapter, the results of the biofilm formation in the four cooling towers are presented over the observation period of ten months in 2019. Water samples were tested for HPC at the incubation temperatures of 22 and 36 °C with the ISO method, for *Legionella* spp. with the ISO method (Lspp_ISO), for *L. pneumophila* with Legiolert (Lp_IDEXX), for *P. aeruginosa* with the ISO method (PA_ISO) and with Pseudalert (PA_IDEXX). The biofilms were tested for HPC (22 and 36 °C) with the ISO method, for *Legionella* spp. and *P. aeruginosa* with modified ISO methods.

In section 3.3.1 the visual assessment of the algae and mosses growth on the cooling tower internals and of the clarity of the cooling water is given over the entire observation period.

The microbiological results of the biofilm composition and water samples are described for each cooling tower in section 3.3.2. It should already be mentioned here that the data sets are too small for reliable statistical statements. Nevertheless, statistical analyses have been carried out in order to be able to derive a trend.

In the two following sections, the microbiological results of the water samples (section 3.3.3) and the biofilm compositions from the stainless steel and polyethylene plates (section 3.3.4) are described comparatively for the four cooling towers. Section 3.3.3 also includes the classification of the *Legionella* results of the water samples tested with the ISO method and Legiolert according to the test and action values of the 42nd BImSchV in subsection 3.3.3.2.

The comparative analyses of each 40[2] *Legionella* and *P. aeruginosa* results tested by ISO and IDEXX methods are described in 3.3.5.

3.3.1 Visual assessment of the four cooling towers

In the Cooling Towers 1, 2 and 4 clear water was recorded throughout the entire observation period. In Cooling Tower 3 the water was clear in February, in the following months it was alternately slightly to moderately turbid with a light brownish coloration. A slight turbidity corresponded to McFarland Standard 0.5 and a moderate turbidity to McFarland Standard 1.

In addition to the visual assessment of the clarity of the water, Table 35 also provides an assessment of algae and mosses growth on the cooling tower internals. The classification is based on the estimation of the average area of the internals covered with algae and mosses.

[2] In total 44 water samples were tested. The water samples taken at the time of installing the plates holding units were analysed with Legiolert and Pseudalert reagents for which the date of expire was exceeded. These results are therefore not included in the comparative analyses, but are shown for completeness in the figures in sections 3.3.2 and 3.3.3.

The description "very few" was chosen for a growth that was less than 10 % of the area. "Few" was used when between 10 and 30 %, "moderate" when 30 to 50 % and "dense" when between 50 to 75 % of the surfaces were covered with algae and mosses. "Very dense" was recorded when more than 75 % of the surfaces were covered with a thick layer of algae and mosses.

Table 35: Visual assessment of the four cooling towers considering the water clarity and the algae growth on internals .
(+): very few, +: few, ++: moderate, +++: dense, ++++: very dense.

	Week #	Δ days	Water clarity				Algae and mosses growth on internals			
			CT1	CT2	CT3	CT4	CT1	CT2	CT3	CT4
Feb 19	8	-	clear	clear	clear	clear	+	+	++	++
Mar 19	12	28	clear	clear	slightly turbid	clear	+	+	++	++
Apr 19	16	28	clear	clear	turbid	clear	+	+	+++	++
May 19	20	27	clear	clear	turbid	clear	+	+	+++	++
Jun 19	24	28	clear	clear	slightly turbid	clear	++	+	++++	++
Jul 19	29	33	clear	clear	turbid	clear	++	(+)	++++	++
Aug 19	32	23	clear	clear	slightly turbid	clear	++	++	++++	++
Sep 19	36	28	clear	clear	turbid	clear	++	+++	++++	++
Sep 19	40	26	clear	clear	turbid	clear	++	+++	++++	++
Oct 19	44	28	clear	clear	turbid	clear	+++	++	++++	++
Nov 19	48	28	clear	clear	slightly turbid	clear	++++	++	+++	++

In Cooling Towers 1 to 3, an increase in vegetation on the internals was observed in the warmer months. Cooling Towers 1 and 2 showed poor algae and moss growth until October. While algae and mosses growth remained moderate from October until November in Cooling Tower 2, a clear increase was observed in Cooling Tower 1. The biocide treatment was deactivated in Cooling Tower 1 between October 7th and November 22nd. In Cooling Tower 3, very dense growth was observed over the summer months. In the cooler months, it was slightly less. In Cooling Tower 4, a moderate vegetation was observed over the entire observation period.

3.3.2 Microbial growth on the stainless steel and polyethylene plates and in water samples

The development of the microbiological composition of the water samples and the biofilms grown on the stainless steel and polyethylene plates from the four cooling towers over the ten-month observation period are presented in the following subsections 3.3.2.1 to 3.3.2.4. Different figures are given for each cooling tower. The first one shows a picture of the plates from the short and long observation interval immediately after the sampling on September 4th 2019. Another figure includes four charts plotting the concentrations for HPC at the incubation temperatures of 22 and 36 °C, for *Legionella* spp. / L. pneumophila and for *P. aeruginosa* from the water samples and stainless steel and polyethylene plates of the short and long intervals. The corresponding concentrations are listed in two tables. Furthermore, the distribution of the *Legionella* species in the water samples and the biofilms of the short and long interval stainless steel and polyethylene plates are shown in five circular diagrams.

The first smears of the plates of the short-term observation interval ("SI") were taken in March, the first smears of the plates of the long-term interval ("LI") in April. At this point, it should be mentioned again that at each removal of plates clean, freshly disinfected plates were installed each time to observe the four weeks short-term interval. The plates of the long-term interval observation were all placed in the cooling towers at the begin of the study in February. The concentrations determined on the short-term plates developed within four weeks, these on the long-term plates developed since February.

3.3.2.1 Cooling Tower 1

Visually clean plates as shown in Figure 40 characterized Cooling Tower 1. The biocide treatment was performed with chlorine dioxide depending on the redox potential except from October 7th to November 22nd 2019. Until the removal in early September, no biofilm was apparent neither on the plates of the long observation interval nor on those of the short interval. From September to November, a very thin layer of a soft, smooth biofilm was present on the entire surface of the plates.

Figure 40: Plates after removal from Cooling Tower 1.
a) stainless steel plate and b) polyethylene plate from the short interval (four weeks), c) stainless steel and d) polyethylene plate from the long interval (28 weeks).

At the day of installing the plates, the HPC for both 22 and 36 °C incubation temperature were higher than in the water samples of the following months as shown in Table 36 and the corresponding upper charts of Figure 41. The 22 °C HPC decreased to 10 cfu/mL by June. The 36 °C HPC reached their minimum below the detection limit already in May. The 22 °C HPC

increased by two log_{10} levels until the investigation in August, decreased slightly until the end of October and increased rapidly by three log_{10} levels until the end of the investigation period during the deactivated biocide treatment. The course of HPC at 36 °C from the water samples is quite similar. Here, too, the concentration increased slowly already from May to August and stagnated between August until the end of September. In the October investigation a decrease of HPC at 36 °C was recorded, followed by a rapid increase by three log_{10} levels. In total, ten of the eleven water samples had a higher concentration of HPC at 22 °C than at 36 °C. In one sample, the same count was determined for both incubation temperatures. The HPC of both incubation temperatures grown on the plates showed significant fluctuations for both plate types and observation intervals, especially in the first half of the ten-month observation period. The HPC at 22 and 36 °C of the biofilm smears from the stainless steel and polyethylene plates tended to reflect the concentration curves of the water samples. In most cases, a parallel increase or decrease of HPC on the plates and in the water samples was observed. The course of the HPC curves was similar for both incubation temperatures, for both plate types and for both test intervals. While the growth of heterotrophic bacteria detectable with Yeast extract agar for both incubation temperatures on the two plate types was characterized by strong increases and decreases of up to three log_{10} levels in the first months of the study, a mostly stagnant growth with a slight increase was observed from July onwards. An exception was the 22 °C HPC of the polyethylene plate of the long-term interval, which was not detected at the beginning of September. In the first investigation three weeks after deactivation of the biocide treatment, a slight decrease was observed for HPC at 22 and 36 °C in the water sample, for HPC at 22 and 36 °C on the polyethylene plates and for 36 °C HPC on the stainless steel plate of the long investigation interval. At 22 °C, HPC increases were observed on the stainless steel plates of both investigation intervals and at 36 °C on the stainless steel plate of the short interval. By the end of the observation period, a significant increase in HPC was observed in the water sample and on both plate types for both investigation intervals at 22 and 36 °C.

For the majority of colonies grown on the Yeast extract agar plates, the same colony morphology types were observed for the water samples, the short-term and the long-term plates of both materials. Detected colonies were not further identified.

In order to assess whether HPC on the different plate types and depending on the test interval differed significantly or could be considered as the same, the laboratory "0.5 log level-rule" was applied. In the Laboratory for Technical Hygiene, this rule is used as an indicator whether microbiological results can be regarded as equal or differ significantly. In fact, the rule is usually applied for results of the same method from the same sample tested in replicates or for results

of the same sampling site tested at different times. Here it is alienated and has trend-recognising property. By definition, results are equal if the difference of the log-transformed results is less than 0.5.

Table 36: Cooling Tower 1 - Microbial concentrations in water samples.
DL: detection limit.

Examination Date	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	*Legionella* spp. ISO [cfu/100 mL]	*L. pneumophila* IDEXX [mpn/100 mL]	*P. aeruginosa* ISO [cfu/100 mL]	*P. aeruginosa* IDEXX [mpn/100 mL]
21.02.2019	9,100	1,500	50	108	<DL	<DL
21.03.2019	210	50	<DL	<DL	10	10
18.04.2019	250	90	55	108	<DL	<DL
15.05.2019	20	<DL	50	23	<DL	<DL
12.06.2019	10	10	<DL	<DL	<DL	<DL
15.07.2019	80	20	<DL	<DL	<DL	<DL
07.08.2019	630	140	<DL	11	20	41
04.09.2019	480	120	<DL	10	30	<DL
30.09.2019	450	140	50	108	<DL	<DL
28.10.2019	290	40	700	108	<DL	<DL
25.11.2019	$3.1*10^5$	$7.3*10^4$	<DL	<DL	80	171

Table 37: Cooling Tower 1 - Microbial concentrations in the biofilms grown on a) stainless steel and b) polyethylene plates.
DL: detection limit.

a)

[cfu/25 cm²]	Short interval observation				Long interval observation			
	HPC 22 °C	HPC 36 °C	*Legionella* spp.	*P. aeruginosa*	HPC 22 °C	HPC 36 °C	*Legionella* spp.	*P. aeruginosa*
21.03.2019	<DL	<DL	5	<DL				
18.04.2019	2,800	1,800	5	<DL	$4.1*10^6$	$4.3*10^5$	<DL	<DL
15.05.2019	900	400	<DL	<DL	<DL	<DL	<DL	<DL
12.06.2019	<DL	<DL	<DL	<DL	9,500	1,600	<DL	<DL
15.07.2019	500	600	<DL	<DL	100	100	<DL	<DL
07.08.2019	$2.9*10^4$	$1.9*10^4$	<DL	<DL	8,000	3,600	<DL	65
04.09.2019	4,400	300	<DL	15	2,800	2,700	<DL	<DL
30.09.2019	$1.3*10^4$	6,900	<DL	275	$1.8*10^4$	$2.1*10^4$	<DL	<DL
28.10.2019	$3.3*10^5$	$1.8*10^5$	10	$2.8*10^4$	$1.3*10^5$	8,300	5	<DL
25.11.2019	$1.4*10^6$	$1.1*10^6$	<DL	<DL	$1.2*10^7$	$9.3*10^6$	<DL	<DL
b)								
21.03.2019	2,900	2,000	<DL	<DL				
18.04.2019	200	4,800	<DL	<DL	$4.6*10^5$	$2.0*10^5$	<DL	<DL
15.05.2019	<DL	<DL	<DL	<DL	200	600	<DL	<DL
12.06.2019	1,900	2,100	<DL	<DL	$2.5*10^4$	$2.0*10^4$	<DL	<DL
15.07.2019	5,700	6,200	<DL	115	200	300	<DL	<DL
07.08.2019	$5.9*10^4$	$3.9*10^4$	<DL	300	1,000	700	<DL	215
04.09.2019	2,100	3,300	<DL	115	<DL	$1.0*10^4$	<DL	<DL
30.09.2019	$2.1*10^4$	5,300	<DL	140	$4.5*10^4$	8,900	<DL	<DL
28.10.2019	$2.1*10^4$	9,400	10	1,100	$2.0*10^4$	4,900	<DL	5
25.11.2019	$2.9*10^6$	$3.6*10^6$	<DL	<DL	$1.2*10^7$	$4.5*10^6$	<DL	<DL

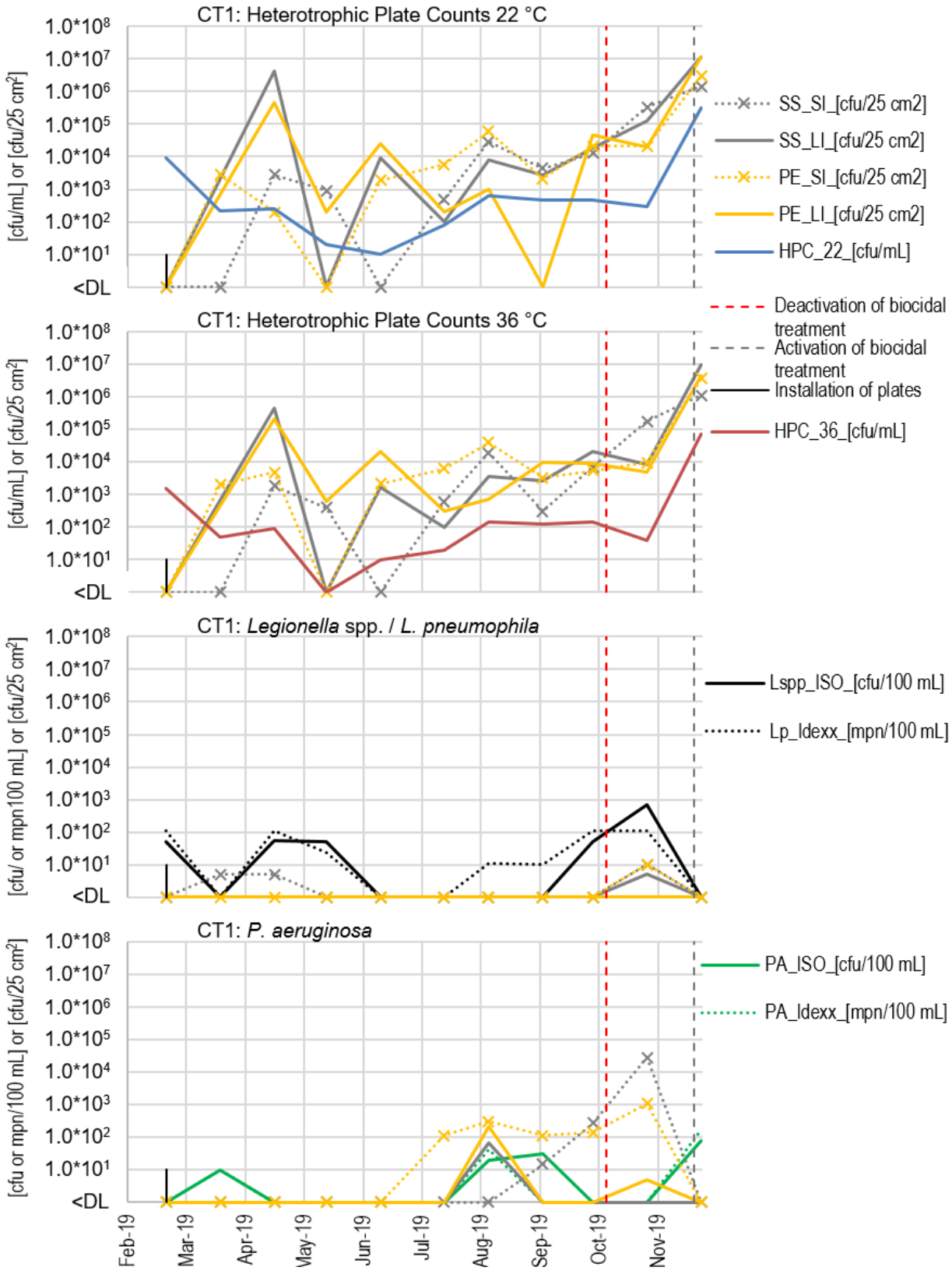


Figure 41: CT1 - Microbial growth on the SS and PE plates and in the water sample.
SS: stainless steel; PE: polyethylene; xx_LI: long interval plate; xx_SI: short interval.

Comparing the 22 °C HPC short interval plates, the counts on the stainless steel plates were higher in four investigations in absolute values and the counts on the polyethylene plates were higher in six investigations. Applying the laboratory's own "0.5 log level-rule" for the HPC at 22 °C polyethylene and stainless steel data sets of the short observation interval revealed that three results were significantly higher on both the stainless steel and the polyethylene plates. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Looking at the long-term plates for HPC at 22 °C, in absolute numbers, four results of stainless steel plates were higher and five results of polyethylene plates were higher. Using the internal laboratory "0.5 log level-rule", four results of the long-term stainless steel plates were significantly higher and one result of the polyethylene plates. Accordingly, four results ranged within a half log level. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Comparing the results of the short-term and long-term stainless steel plates for HPC at 22 °C, five results were higher on the plates of the short-term interval and four of the long-term interval. Using the "0.5 log level-rule", three results of the short-term plates and three of the long-term plates were significantly higher. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly. For the polyethylene plates, in total, four results of the short and five results of the long interval were higher. Using the "0.5 log level-rule", three results of the short-term plates and four of the long-term plates were significantly higher. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly.

Regarding the HPC at 36 °C grown on the short-term plates, three results were higher from the stainless steel plates, two of which were significantly higher according to the “0.5 log level” laboratory rule compared to the polyethylene results of the same sampling date. On the polyethylene plates, seven 36 °C-HPC results were higher, five of which were significantly higher than the results of the stainless steel plates. Three pairs of results on the two material types of short-time plates ranged within half a log level. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Considering the HPC at 36 °C of the long-term plates, of the five higher counts on the stainless steel plates, one result was significantly higher. Of the four higher results on the polyethylene plates, three were significantly higher according to the "0.5 log level-rule". The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Looking at the HPC at 36 °C grown on the stainless steel plates of the short and long interval, four results were higher at the short interval and five at the long interval. Four results each

were significantly higher for the two test intervals according to the "0.5 log level-rule". One pair of results differed by less than half a log level-rule. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly. When comparing the polyethylene plates, three pairs of results showed HPC at 36 °C higher on the short interval plates (two of them were significantly higher according to the "0.5 log level-rule") and six on the plates of the long interval (three of them were significantly higher). The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly.

As mentioned above, in the water samples 22 °C-HPC were higher in ten of eleven samples. Applying the “0.5 log level-rule” in nine samples the HPC at 22 °C were significantly higher than at the incubation temperature of 36 °C. The performance of the Wilcoxon signed-rank test indicated that these data sets differed significantly comparing the calculated W-value with the N-value at a significance level at 5.0 %.

In the water samples legionellae were detected in low concentrations with both methods. Using the ISO method, 50 to 55 cfu *L. pneumophila*/100 mL were detected in February, April and May. With Legiolert, two times higher *L. pneumophila* concentrations were obtained in these months with 108 mpn/100 mL and in May in a lower concentration of 23 mpn/100 mL. Legionellae were not detected between June and September using the ISO method. One non-*pneumophila Legionella species* was detected with the ISO method in September and October. In October, after the biocide deactivation, the highest concentration in the observation period was recorded at 700 cfu/100 mL. By Legiolert, *L. pneumophila* was not detected in June and July. In August and September, concentrations of 10^1 mpn/100 mL were determined. In September and October, concentrations of 10^2 mpn/100 mL were obtained. After reactivation of the biocide treatment, legionellae were not detected in November with both methods.

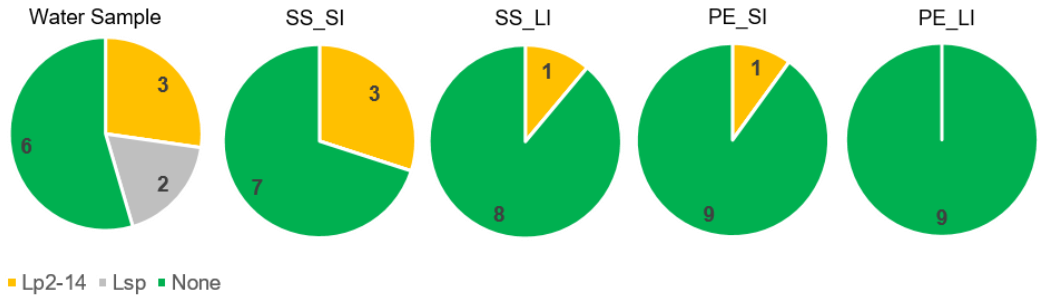


Figure 42: CT1 - Distribution of *Legionella* strains in water samples (ISO method; n = 11) and on the different short-term (n = 10) and long-term (n = 9) plates.
SS: stainless steel; PE: polyethylene; xx-LI: long interval plate; xx_SI: short interval.

The distribution of detected *Legionella species* and *L. pneumophila* strains is shown in Figure 42. Legionellae were rarely detected on the short- and long-term plates. On the stainless steel

plates of the short interval *L. pneumophila* was determined in March, April and October at very low concentrations of 5 to 10 cfu/25 cm^2. On the long-term stainless steel plates, *L. pneumophila* was only determined in October in also low concentrations of 5 cfu/25 cm^2. *L. pneumophila* was detected on one polyethylene plate of the short observation interval after the plate removal in October. On the polyethylene plates of the long interval legionellae were not found over the entire observation period. Neither in the water samples nor on any of the plates legionellae co-contaminations were observed.

Due to the small number of legionellae and *P. aeruginosa* detections, neither the "0.5 log level-rule" nor statistical tests were applied.

In the water samples, *P. aeruginosa* was detected in four water samples using the ISO method in March, August, early September and November. By Pseudalert the bacterium showed positive signals in three samples in March, August and November. The highest concentration of 171 mpn/100 mL was determined with Pseudalert in November. All other concentrations for both the ISO and the IDEXX methods ranged below 100 cfu or mpn/100 mL. *P. aeruginosa* was detected on the stainless steel plates of the short interval at three removals in September and October in continuously increasing concentrations from 15 to $2.8 * 10^4$ cfu/25 cm^2. Only in August, *P. aeruginosa* was recorded on the long-term stainless steel plates at a concentration of 65 cfu/25 cm^2. Between July and late September, the microorganism grew on the polyethylene plates of the short observation interval in concentrations from 100 to 300 cfu/25 cm^2. The peak was reached in October at 1,100 cfu/25 cm^2. After the biocide reactivation in November *P. aeruginosa* was not detected on the polyethylene plate of the short interval. On the polyethylene plates of the long interval, *P. aeruginosa* was sporadically found in August and October.

3.3.2.2 Cooling Tower 2

The plates of the ozone-treated Cooling Tower 2 had a distinctive appearance. A thin layer of brown-black, tough, crusty deposits, temporarily iron-containing brown-red sludge and the occurrence of snails and small crustaceans characterized the plates in Cooling Tower 2 (see Figure 43). On both the short-term and the long-term plates, the iron-bearing sludge was observed during the sampling periods from March respectively from April to May. Snails and crustaceans were recorded on the plates from March/April to October.

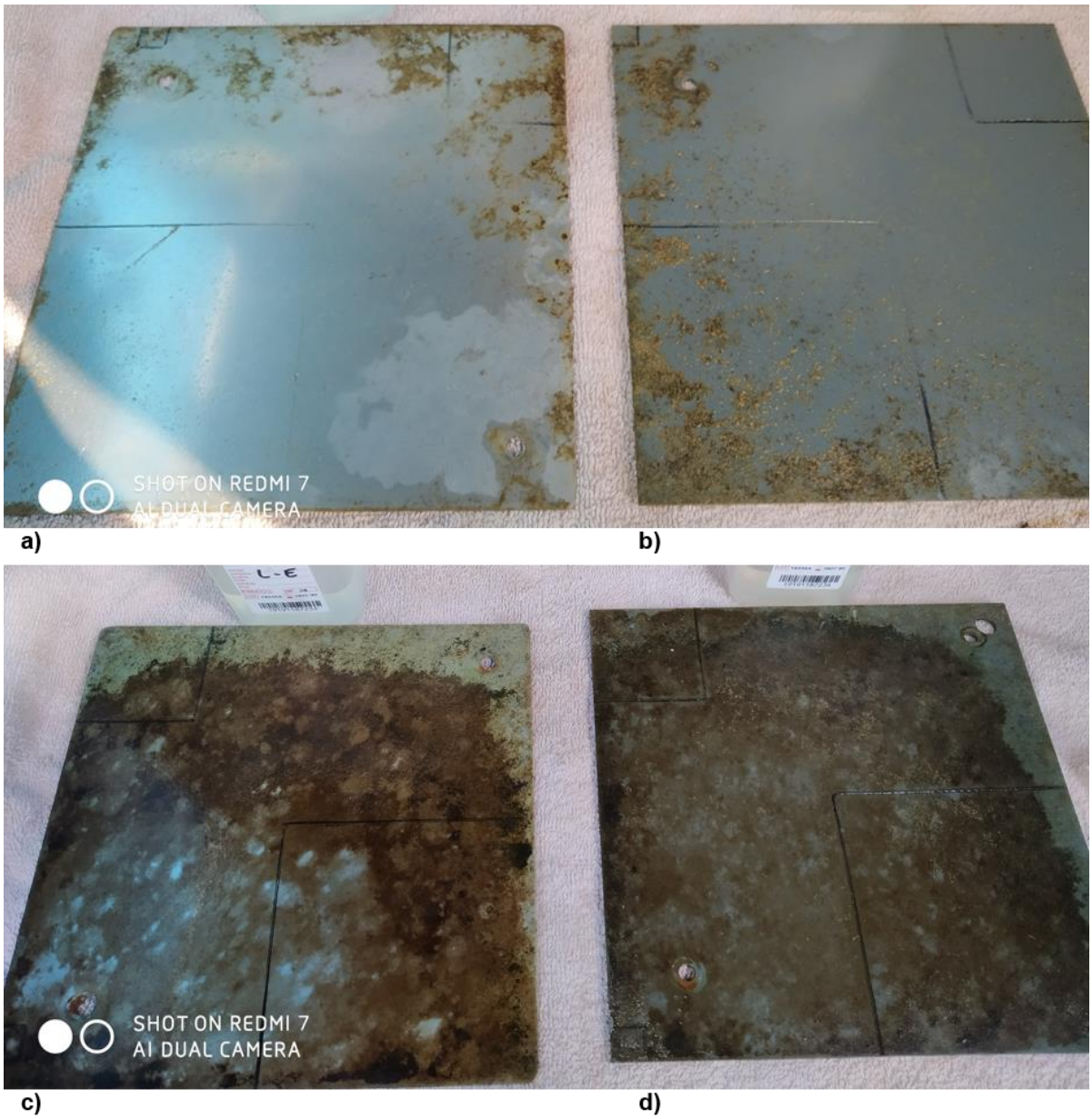


Figure 43: Plates after removal from Cooling Tower 2.
a) stainless steel plate and b) polyethylene plate from the short interval (four weeks), c) stainless steel and d) polyethylene plate from the long interval (28 weeks).

On the short-term plates, the tough biofilm was less from March to May than from June to September. In October and November, it was a very thin layer. On the long-term plates, the biofilm increased continuously from April until the end of September and decreased in October and November. Nevertheless, the biofilm formed a thin layer on the plates of both investigation intervals. The crusty, tough biofilm was difficult to scrape off and left a blackish discoloration on the stainless steel plates.

In Cooling Tower 2, the curves of the microbiological parameters in the water samples were all marked by fluctuations.

As shown in Figure 44, the HPC curves of both incubation temperatures were nearly congruent. The calculated concentrations shown in Figure 44 are listed in Table 38 and Table 39. Their curves started with the February investigation between 2,000 and 3,000 cfu/mL, decreased slightly until April and then declined sharply to the detection limit (22 °C) or below (36 °C) in the May investigation. Within two months, the HPC increased rapidly to 71,900 cfu/mL in July. In the August investigation, the HPC were reduced by two $\log_{10}$ levels. At the end of September, peaks at 10,600 cfu/mL (22 °C) and 5,100 cfu/mL (36 °C) were observed after a special dosage at September 26th. In October, the HPC dropped to the August values and hardly rose until the end of the investigation period in November. In eight of the eleven water samples, higher values were determined for HPC at 22 °C incubation temperature. In two samples, the counts were the same for both incubation temperatures.

The HPC curves of both incubation temperatures and plate types were very similar for the respective observation interval (see Figure 44). The HPC of the long interval increased to over 10^5 cfu/25 cm^2 within eight to twelve weeks. Until the end of September, HPC increased almost continuously to 10^7 cfu/25 cm^2, but with a slight decrease here and there. With the special biocide dosage, a decrease in HPC at 22 °C on both plate types and HPC at 36 °C on the stainless steel plate was observed by about one log level. The special biocide dosage seemed to have no effect on HPC at 36 °C on the polyethylene plate. During the short observation interval, concentrations of 10^4 to 10^6 cfu/25 cm^2 were always detected on both plate types for HPC at 22 °C between May and November. Already in March, almost 10^6 cfu/25 cm^2 were determined on the HPC-22 °C polyethylene plate. In April, a lower concentration by a factor of 1,000 was found. On the HPC-22 °C-stainless steel plates, markedly lower concentrations were determined in March and April than in the rest of the year. The HPC at 36 °C of the short investigation interval showed concentrations in the range of 10^3 cfu/25 cm^2 in the first investigation in March on both plate types. Between April and November, counts between 10^4 and 10^6 cfu/25 cm^2 were also detected.

For the majority of colonies grown on the Yeast extract agar plates, the same colony morphology types were observed in the water samples for both incubation temperatures. In the first half of the observation period, the same colony morphology types of the short-term and the long-term plates of both materials were recorded. In the second half, more colonies grown on the agar plates of the long interval differed in their appearance from those of the short interval. Detected colonies were not further identified.

In order to assess whether the HPC of the different plate types and test intervals differed significantly or could be considered as the same, the laboratory "0.5 log level-rule" was applied.

Comparing the 22 °C-HPC short-interval plates, the counts of the stainless steel plates were higher in seven investigations in absolute values and the counts of the polyethylene plates were higher in three investigations. Using the laboratory's own "0.5 log level-rule", it was determined that three results for HPC at 22 °C from the stainless steel plates were significantly higher and one result from the polyethylene plates was significantly higher. Hence, the results differed less than a half log level in seven investigations. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Looking at the long-term plates for HPC at 22 °C, in absolute numbers, four results from stainless steel plates were higher and five results from the polyethylene plates were higher. Using the internal laboratory "0.5 log level-rule", one result of the long-term stainless steel plates and two results of the polyethylene plates were significantly higher. Accordingly, six results ranged within a half log level. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Comparing the results of the short-term and long-term stainless steel plates for HPC at 22 °C, three results were higher from the plates of the short-term interval and six results were higher for the long-term interval. Using the "0.5 log level-rule", no result of the short-term plates and five of the long-term plates were significantly higher. Four results can be regarded as equally according to the “0.5 log level-rule”. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly. For the polyethylene plates, all nine results of the long interval were higher. Using the "0.5 log level-rule", eight results were significantly higher. The performance of the Mann-Whitney-U-test indicated that these data sets differed significantly.

Regarding the heterotrophic bacteria grown at the incubation temperature of 36 °C on the short-term plates, four results from the stainless steel plates were higher, three of which were significantly higher according to the laboratory rule compared to the corresponding results from the polyethylene plates. Six results of the polyethylene plates were higher for HPC at 36 °C, of whose one result was significantly higher compared to the corresponding one from the stainless steel plate. Seven results from the two types of short-time plates differed less than a half log level. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Table 38: Cooling Tower 2 - Microbial concentrations in water samples.
DL: detection limit.

Examination Date	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	*Legionella* spp. ISO [cfu/100 mL]	*L. pneumophila* IDEXX [mpn/100 mL]	*P. aeruginosa* ISO [cfu/100 mL]	*P. aeruginosa* IDEXX [mpn/100 mL]
21.02.2019	2,700	2,300	25	108	20	20
21.03.2019	700	430	85	90	<DL	10
18.04.2019	650	330	<DL	<DL	<DL	<DL
15.05.2019	<DL	10	5	<DL	<DL	30
12.06.2019	60	60	<DL	<DL	<DL	<DL
15.07.2019	$7.2*10^4$	$2.2*10^4$	350	<DL	11	41
07.08.2019	200	70	5	11	<DL	<DL
04.09.2019	2,800	1,770	100	47	<DL	41
30.09.2019	$1.1*10^4$	5,110	<DL	<DL	<DL	<DL
28.10.2019	180	70	5	<DL	<DL	<DL
25.11.2019	330	180	<DL	11	<DL	<DL

Table 39: Cooling Tower 2 - Microbial concentrations in the biofilms grown on a) stainless steel and b) polyethylene plates.
DL: detection limit.

a)

[cfu/25 cm²]	Short interval observation				Long interval observation			
	HPC 22 °C	HPC 36 °C	*Legionella* spp.	*P. aeruginosa*	HPC 22 °C	HPC 36 °C	*Legionella* spp.	*P. aeruginosa*
21.03.2019	1,800	3,300	5	<DL				
18.04.2019	7,700	$4.0*10^4$	<DL	<DL	3,300	$2.5*10^5$	<DL	<DL
15.05.2019	$1.4*10^5$	$2.2*10^5$	<DL	<DL	$2.3*10^5$	$3.8*10^5$	<DL	<DL
12.06.2019	$1.4*10^5$	$1.1*10^5$	<DL	<DL	$5.4*10^4$	$7.4*10^4$	<DL	<DL
15.07.2019	$1.2*10^6$	$1.2*10^6$	<DL	$4.3*10^4$	$9.1*10^5$	$2.7*10^5$	<DL	<DL
07.08.2019	$1.1*10^5$	$1.6*10^5$	<DL	250	$4.1*10^5$	$1.1*10^6$	<DL	<DL
04.09.2019	$1.7*10^5$	$8.2*10^5$	<DL	1,100	$1.1*10^6$	$1.5*10^6$	<DL	1,300
30.09.2019	$1.3*10^6$	$7.9*10^5$	<DL	$1.5*10^4$	$9.3*10^6$	$9.0*10^6$	<DL	500
28.10.2019	$1.5*10^5$	$1.5*10^5$	<DL	<DL	$4.7*10^6$	$4.8*10^6$	<DL	<DL
25.11.2019	$2.4*10^5$	$5.2*10^5$	<DL	7,400	$1.4*10^6$	$5.3*10^5$	<DL	<DL
b)								
21.03.2019	$7.2*10^5$	8,900	<DL	<DL				
18.04.2019	690	$2.0*10^5$	10	<DL	$9.9*10^4$	$5.9*10^4$	<DL	<DL
15.05.2019	$3.3*10^4$	$1.9*10^5$	<DL	<DL	$1.3*10^5$	$3.1*10^5$	<DL	<DL
12.06.2019	$8.9*10^4$	$2.0*10^5$	<DL	<DL	$9.9*10^4$	$1.3*10^5$	<DL	<DL
15.07.2019	$7.6*10^5$	$1.8*10^5$	<DL	1,900	$4.*10^6$	$3.5*10^5$	<DL	<DL
07.08.2019	$1.1*10^5$	$1.9*10^5$	<DL	2,500	$4.7*10^5$	$1.5*10^6$	<DL	<DL
04.09.2019	$6.0*10^4$	$6.0*10^4$	<DL	100	$7.7*10^5$	$2.4*10^6$	100	500
30.09.2019	$1.6*10^6$	$1.2*10^6$	<DL	$2.1*10^4$	$1.1*10^7$	$8.3*10^6$	<DL	<DL
28.10.2019	$2.5*10^5$	$2.2*10^5$	<DL	<DL	$1.3*10^6$	$3.2*10^6$	<DL	<DL
25.11.2019	$6.2*10^4$	$1.1*10^5$	<DL	500	$1.1*10^6$	$4.5*10^6$	<DL	<DL

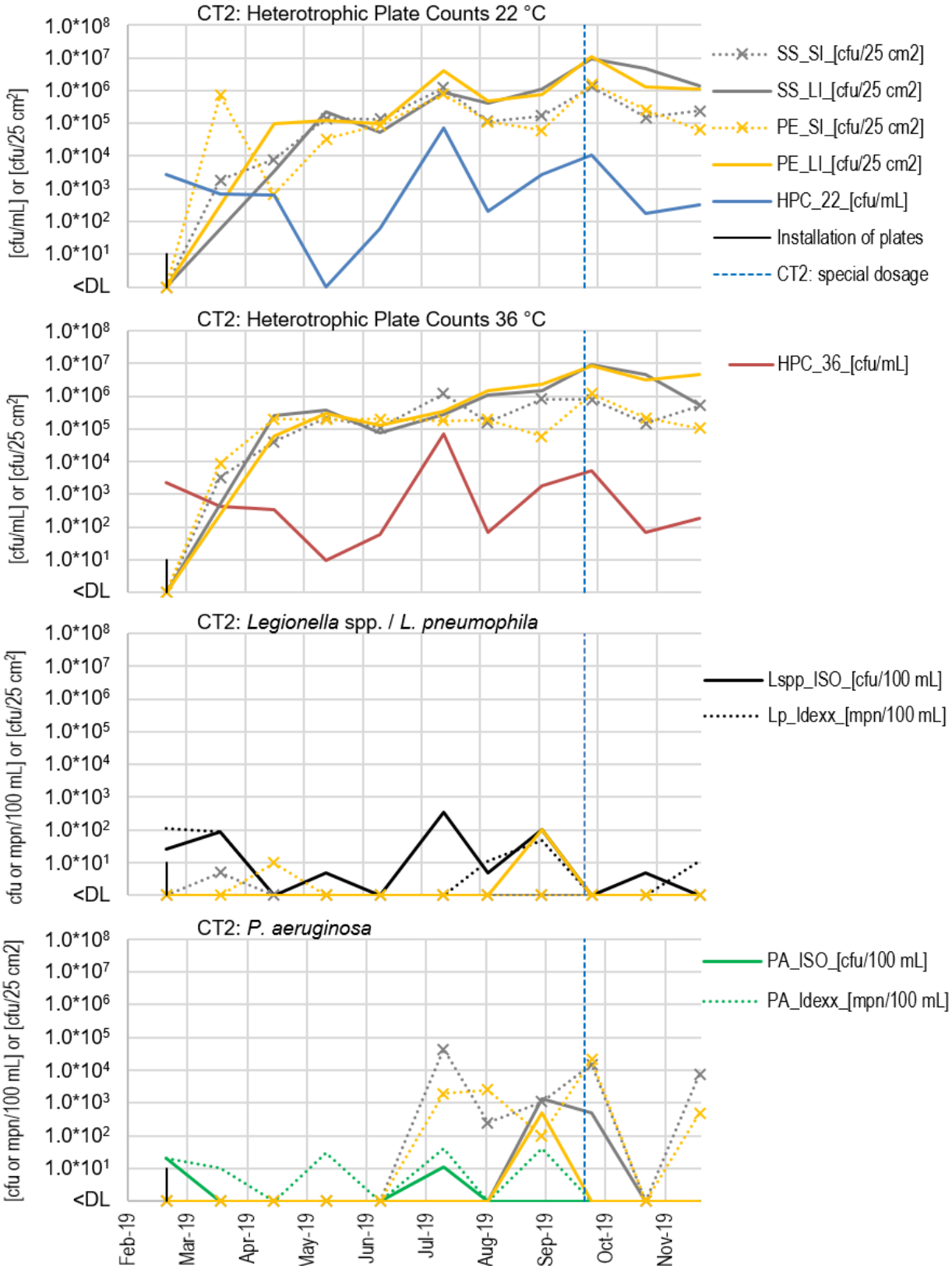


Figure 44: CT2 - Microbial growth on the SS and PE plates and in the water sample.
SS: stainless steel; PE: polyethylene; xx-LI: long interval plate; xx_SI: short interval.

Looking at the HPC at 36 °C from the long-term plates, of the four higher counts on the stainless steel plates, one result was significantly higher according to the "0.5 log level-rule". Of the five higher results from the polyethylene plates, one was significantly higher according to the "0.5 log level-rule". The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Considering the results for HPC at 36 °C grown on the stainless steel plates of the short and long interval, two results were higher from the short interval plates and seven from the long interval plates. One result was significantly higher for the short interval and four results from the long-term plates were significantly higher according to the "0.5 log level-rule". Results from four removal dates differed by less than a half log level. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly. When comparing the short-term and long-term polyethylene plates, two results for HPC at 36 °C were higher on the short interval plates (one significantly according to the "0.5 log level-rule") and seven results of the plates from the long interval (five significantly). Hence, results of three results ranged within a half log level. The performance of the Mann-Whitney-U-test indicated that these data sets differed significantly.

As mentioned above, HPC at 22 °C incubation temperature were higher in eight of eleven water samples compared to HPC at 36 °C. HPC at 36 °C was higher in one sample. Applying the “0.5 log level-rule” no result for the HPC at 22 °C was significantly higher than the count at 36 °C. In one sample the count for 22 °C was below the detection limit and the count for 36 °C corresponded to the detection limit of 10 cfu/mL, that the "log 0.5-rule" indicated the results were significantly different. The performance of the Wilcoxon signed-rank test indicated that these data sets differed significantly.

The ISO-*Legionella* spp. curve was subject to stronger fluctuations than the Legiolert-*L. pneumophila* curve as shown in Figure 44. Using the ISO method, concentrations below 100 cfu/100 mL were recorded in February and March. *Legionella* spp. were not detected in the April investigation, the detection limit was reached in May, and again Legionellae were absent in June. The maximum concentration determined with the ISO method in Cooling Tower 2 was 350 cfu/100 mL in the July investigation. In the investigations of the remaining months, concentrations were always recorded which corresponded to the detection limit. In three water samples, co-contaminations of *Legionella* strains were detected. In February, March and July, co-existing strains of *L. pneumophila* serogroup 1 and from serogroup range 2 - 14 were found. In July, an additional *Legionella species* was identified which did not show a positive reaction in the latex agglutination test. Even by Legiolert, concentrations around 100 mpn/100 mL were detected in February and March. No *L. pneumophila* were detected by Legiolert in the investigations from April to July. From July onwards, the curve increased and at the beginning of

September, a peak of 47 mpn/100 mL was detected. *L. pneumophila* was not detected in the following two investigations. In the sample of November, a concentration equal to the detection limit was determined by Legiolert.

The distribution of *Legionella* strains in water samples and on the plates is shown in Figure 45.

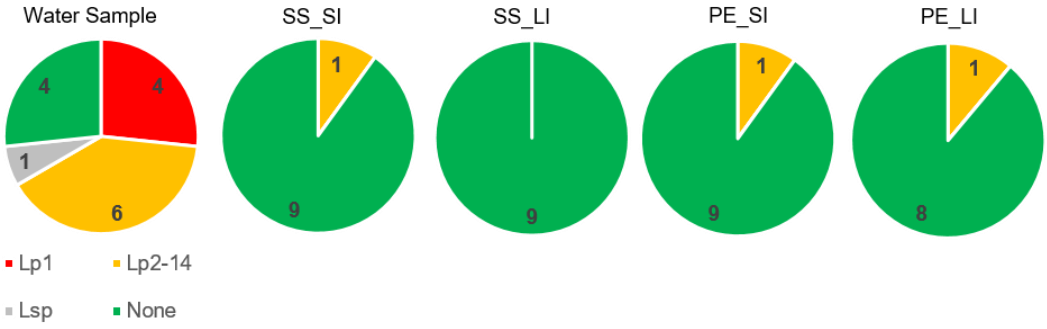


Figure 45: CT2 - Distribution of *Legionella* strains in water samples (ISO method; n = 11) and on the different short-term (n = 10) and long-term (n = 9) plates.
SS: stainless steel; PE: polyethylene; xx-LI: long interval plate; xx_SI: short interval.

In Cooling Tower 2, *Legionella* spp. was detected on the plates the least frequently of all cooling towers. On the plates of the short interval, *Legionella* spp. was detected only on the stainless steel plate in March in a low concentrations of 5 cfu/25 cm^2 on the and polyethylene plate at 10 cfu/25 cm^2 in April. On a single long-term polyethylene plate *Legionella* were detected at 100 cfu/25 cm^2 in early September. On the stainless steel plates of the long observation interval *Legionella* was not found at all. All grown *Legionella* colonies from the biofilm suspensions were identified as *L. pneumopihla* from serogroups range 2 - 14.

Due to the small number of legionellae and *P. aeruginosa* detections, neither the "0.5 log level-rule" nor statistical tests were applied.

P. aeruginosa was detected two times with the ISO method in February and July and five times with Pseudalert, for both methods in low concentrations below 100 cfu or mpn/100 mL. On both plate types of the short and of the long observation interval, *P. aeruginosa* was not detected in the spring months. On the two plate types of the short interval, the bacterium was detected between July and November, excluding October, in varying concentrations ranging from 10^2 to $4.3 * 10^4$ cfu/25 cm^2. Regarding the long interval, *P. aeruginosa* was found on the stainless steel plates in both September investigations and once on polyethylene in early September.

3.3.2.3 Cooling Tower 3

The biofilm grown on the stainless steel plates in chlorine-treated Cooling Tower 3 was characterized by its thickness and the presence of algae and its greasy, fluffy consistency (see Figure 46). The plates of the short-term monitoring were always unevenly covered. During the colder months, free areas predominated. During the warmer months, overgrown areas were predominant. On the long-term plates, an increase in biofilm thickness was observed with each month until the end of September. In the last two months of the study the biofilm thickness did not increase, but decreased slightly.

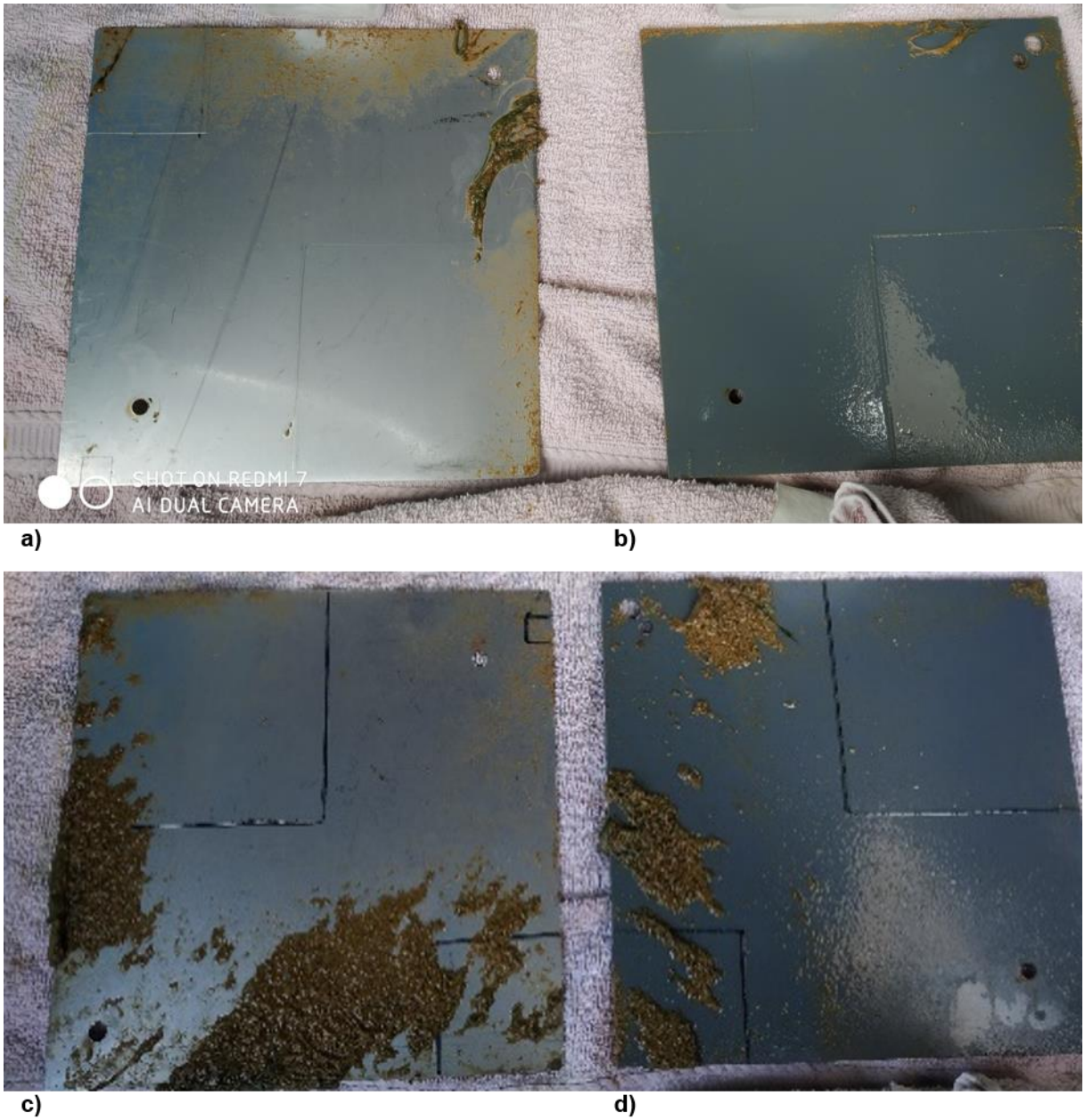


Figure 46: Plates after removal from Cooling Tower 3.
a) stainless steel plate and b) polyethylene plate from the short interval (four weeks), c) stainless steel and d) polyethylene plate from the long interval (28 weeks).

In Cooling Tower 3, fluctuating curves were observed for each parameter in the water samples, but the curves did not oscillate in the same rhythm (see Figure 47). On the short-term and long-term plates, a tendency of stable growth with a slight increase in concentration was observed over the entire investigation period of HPC for both incubation temperatures. All parameters were detected frequently. The corresponding concentrations shown in Figure 47 are listed in Table 40 and Table 41.

Regarding the water samples, the HPC curves of the incubation temperatures of 22 and 36 °C were almost congruent and oscillated by up to three $\log_{10}$ levels. From February to May, the HPC decreased by two $\log_{10}$ levels. From May to July, concentrations increased by almost two $\log_{10}$ levels, and fell by the same amount by the end of August. The HPC at 22 °C curve rose by only one $\log_{10}$ level between the August sampling and the one in early September and then fell by one $\log_{10}$ level until sampling in November. The HPC 36 °C curve rose again by two $\log_{10}$ levels in September, decreased by one $\log_{10}$ level and then showed a tendency to rise again. Of the eleven water samples tested, the same concentration was determined in one sample for both incubation temperatures. In four samples, the HPC at 22 °C was higher and in six samples the HPC at 36 °C.

The HPC graphs of the long-term interval showed a very similar course for both plate types, the incubation temperatures considered separately. The most significant increase occurred in the first eight weeks over four to five $\log_{10}$ levels. For the 22 °C-HPC, stagnating growth with only a slight increase was observed for both plate types until the August examination. At the beginning of September, a reduction of HPC at 22 °C by one $\log_{10}$ level was observed. Subsequently, a further increase in HPC at 22 °C was recorded on both long-term plate types up to 10^7 cfu/25 cm^2. For the long-term plates, the HPC at 36 °C showed a stair-like increase of growth. In the October investigation peak values of more than 10^8 cfu/25 cm^2 were recorded.

On the short-term plates, HPC of both incubation temperatures showed slightly lower concentrations below 10^5 cfu/25 cm^2 in the spring months compared to the other months. Except for two 22 °C polyethylene plates in July and early September, concentrations between 10^5 and 10^6 cfu/25 cm^2 were found in most cases. At the end of September, HPC at 36 °C from the stainless steel plate even exceeded the 10^6 mark.

For the majority of colonies grown on the Yeast extract agar plates, the same colony morphology types were observed in the water samples for both incubation temperatures. In the first half of the observation period, the same colony morphology types of the short-term and the long-term plates of both materials were recorded. In the second half, more colonies grown on the agar plates of the long interval differed in their appearance from those of the short interval. Detected colonies were not further identified.

Table 40: Cooling Tower 3 - Microbial concentrations in water samples
DL: detection limit; n: not evaluable.

Examination Date	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	*Legionella* spp. ISO [cfu/100 mL]	*L. pneumophila* IDEXX [mpn/100 mL]	*P. aeruginosa* ISO [cfu/100 mL]	*P. aeruginosa* IDEXX [mpn/100 mL]
21.02.2019	$1.9*10^4$	9,800	110	90	400	241
21.03.2019	690	330	400	108	220	134
18.04.2019	340	370	<DL	<DL	520	86
15.05.2019	20	40	310	1.062	2.380	1.223
12.06.2019	230	250	600	108	n	3.255
15.07.2019	$1.3*10^4$	$1.0*10^4$	3.700	386	1.300	1.722
07.08.2019	530	620	1.300	155	n	1.553
04.09.2019	110	20	<DL	<DL	<DL	<DL
30.09.2019	1.900	8.000	300	520	n	9.804
28.10.2019	860	530	250	108	<DL	2.187
25.11.2019	300	1.650	1.800	108	n	203

Table 41: Cooling Tower 3 - Microbial concentrations in the biofilms grown on a) stainless steel and b) polyethylene plates.
DL: detection limit.

a)

[cfu/25 cm²]	Short interval observation				Long interval observation			
	HPC 22 °C	HPC 36 °C	*Legionella* spp.	*P. aeruginosa*	HPC 22 °C	HPC 36 °C	*Legionella* spp.	*P. aeruginosa*
21.03.2019	4,300	6,500	<DL	<DL				
18.04.2019	$3.9*10^4$	$7.5*10^4$	5	<DL	$3.2*10^5$	$1.6*10^5$	300	95
15.05.2019	$4.4*10^4$	$1.4*10^5$	<DL	620	$4.4*10^5$	$1.3*10^5$	500	1,600
12.06.2019	$1.0*10^6$	$1.3*10^6$	200	600	$1.3*10^6$	$4.9*10^6$	800	1,200
15.07.2019	$2.2*10^5$	$2.6*10^5$	300	400	$7.5*10^5$	$1.8*10^5$	1.600	1,600
07.08.2019	$4.5*10^5$	$2.2*10^5$	<DL	$2.6*10^4$	$7.7*10^6$	$4.8*10^7$	2.200	1,200
04.09.2019	$2.6*10^5$	$3.0*10^5$	1,500	3,700	$2.1*10^5$	$9.4*10^6$	$2.2*10^4$	500
30.09.2019	$4.5*10^5$	$6.9*10^6$	1,000	$1.2*10^4$	$1.0*10^6$	$2.3*10^7$	$1.5*10^4$	9,700
28.10.2019	$2.2*10^5$	$1.7*10^5$	400	8,300	$1.0*10^7$	$4.2*10^8$	8,200	3,300
25.11.2019	$4.5*10^5$	$8.3*10^5$	500	2,900	$3.1*10^7$	$2.0*10^7$	2,900	2,700
b)								
21.03.2019	$6.3*10^4$	$2.3*10^5$	100	50				
18.04.2019	$1.6*10^4$	$2.9*10^5$	35	<DL	$6.0*10^4$	$4.2*10^5$	<DL	105
15.05.2019	$1.5*10^4$	$8.5*10^4$	100	260	$4.5*10^5$	$1.2*10^6$	500	3,100
12.06.2019	$1.2*10^6$	$2.3*10^5$	100	$1.5*10^4$	$2.4*10^5$	$2.4*10^6$	100	300
15.07.2019	$1.0*10^4$	$3.9*10^5$	300	1,400	$2.0*10^6$	$4.8*10^5$	1,400	3,000
07.08.2019	$3.6*10^5$	$1.2*10^5$	100	$1.4*10^4$	$2.7*10^6$	$4.8*10^7$	$1.0*10^5$	2,300
04.09.2019	6,900	$1.6*10^5$	800	<DL	$1.2*10^5$	$3.8*10^6$	6,400	200
30.09.2019	$1.6*10^6$	$1.8*10^6$	300	7,500	$1.9*10^6$	$2.7*10^7$	<DL	9,000
28.10.2019	$2.1*10^5$	$1.8*10^6$	600	1,800	$1.9*10^6$	$1.7*10^8$	600	2,700
25.11.2019	$1.9*10^5$	$3.8*10^5$	100	45	$7.2*10^6$	$1.7*10^7$	4,600	2,700

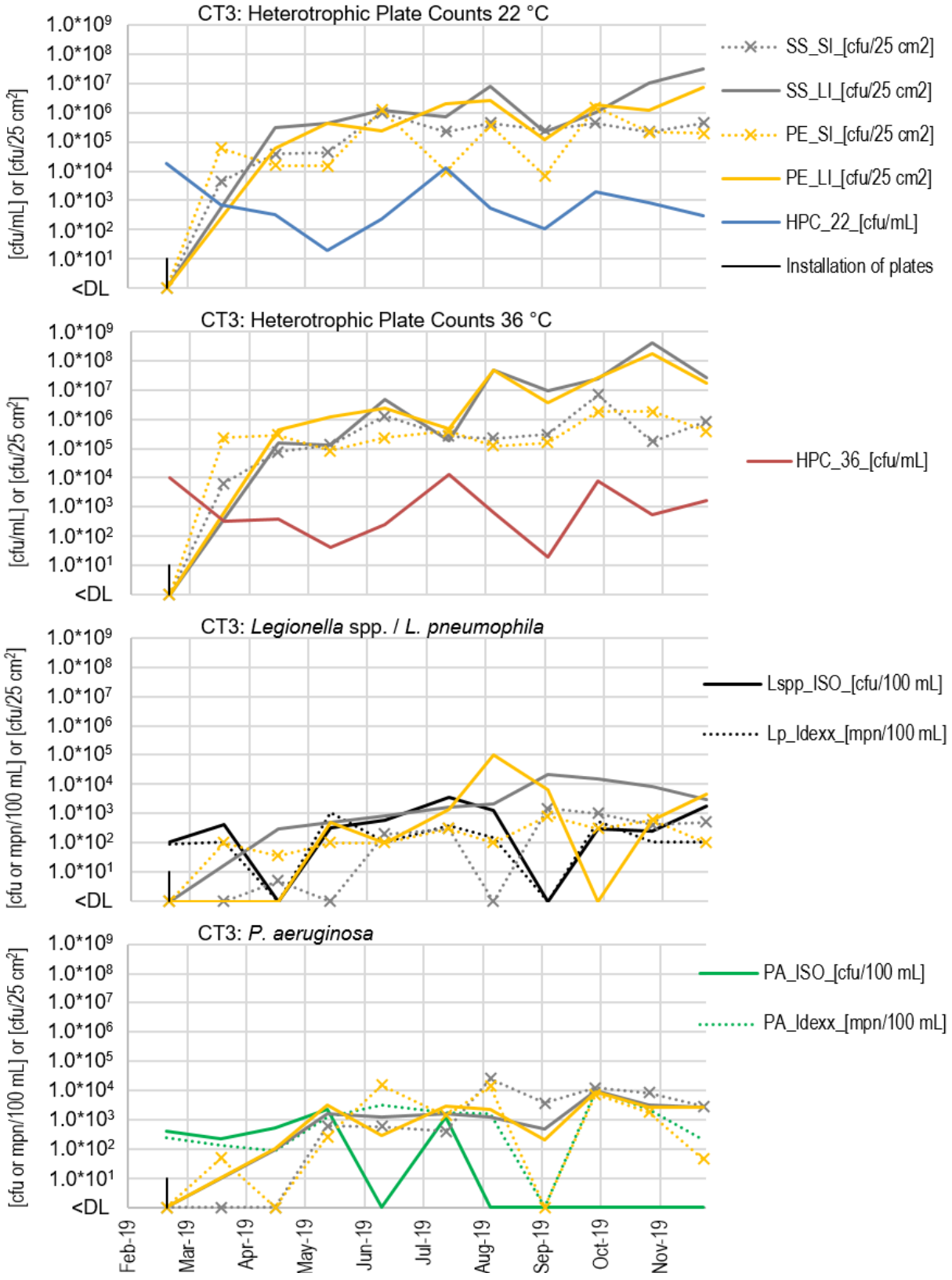


Figure 47: CT3 - Microbial growth on the SS and PE plates and in the water samples.
SS: stainless steel; PE: polyethylene; xx-LI: long interval plate; xx_SI: short interval.

In order to assess whether the HPC of the different plate types and test intervals differed significantly or could be considered as the same, the laboratory "0.5 log level-rule" was applied.

Comparing the heterotrophic bacteria grown at 22 °C incubation temperature on the short-interval plates, the counts of the stainless steel plates were higher in seven investigations in absolute values and the counts of the polyethylene plates were higher in three investigations. Using the laboratory's own "0.5 log level-rule" it was determined that two results for HPC at 22 °C from each the stainless steel and the polyethylene plates were significantly higher compared to the corresponding result of the other plate type. Hence, the results differed less than a half $\log_{10}$ level in six investigations. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Regarding the long-term plates for HPC at 22 °C, in absolute numbers, six results of the stainless steel plates were higher and three results from the polyethylene plates were higher. Using the internal laboratory "0.5 log level-rule", four results of the long-term stainless steel plates were significantly higher and no result of the polyethylene plates was significantly higher. Accordingly, five results ranged within a half $\log_{10}$ level. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Comparing the results of the short-term and long-term stainless steel plates for HPC at 22 °C, one result was higher from the plates of the short-term interval and eight results were higher for the long-term interval. Using the "0.5 log level-rule", no result of the short-term plates, but six of the long-term plates were significantly higher. Three results can be regarded as equally according to the “0.5 log level-rule”. The performance of the Mann-Whitney-U-test indicated that these data sets differed significantly. For the polyethylene plates, one result was higher from the plates of the short-term interval and eight results were higher for the long-term interval. Using the "0.5 log level-rule", one result of the short interval and seven results of the long interval were significantly higher. Accordingly, the results of one removal date can be regarded as equally. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly.

Regarding the HPC at 36 °C grown on the short-term plates, six results from the stainless steel plates were higher. For the polyethylene plates, four results were higher for HPC at 36 °C incubation temperature. Applying the laboratory “0.5 log level-rule” two results from the stainless steel plates were significantly higher compared to the corresponding short-term interval polyethylene steel plate results. Three counts of the polyethylene plates were significantly higher. Five results from the two types of short-time plates differed less than a half $\log_{10}$ level. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Looking at the HPC at 36 °C from the long-term plates, of the four higher counts on the stainless steel plates, no result was significantly higher. Of the four higher results from the polyethylene plates, one was significantly higher according to the "0.5 log level-rule". Accordingly, eight samples differed less than a half log_{10} level. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Comparing the results for HPC at 36 °C grown on the stainless steel plates of the short and long interval, two results were higher from the short interval plates and seven from the long interval plates. No result was significantly higher for the short interval, but six results from the long-term plates were significantly higher according to the "0.5 log level-rule". Results from three plate investigations differed by less than a half log_{10} level. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly. Regarding the short-term and long-term polyethylene plates all nine results of the plates from the long interval were higher and seven of them were significantly higher compared to the result of the short-interval from the same removal date. Hence, results of two results ranged within a half log_{10} level. The performance of the Mann-Whitney-U-test indicated that these data sets differed significantly.

As mentioned above, HPC at 22 °C incubation temperature were higher in four of eleven water samples compared to HPC at 36 °C. HPC at 36 °C were higher in six samples. In one sample, the count was the same for 22 and 36 °C. Applying the “0.5 log level-rule” one result for the HPC at 22 °C was significantly higher than the count for 36 °C and two results were significantly higher for HPC at 36 °C. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

In the water samples, the legionellae concentration dropped in spring and minima were recorded for both methods in April. During sampling in May, the highest concentration of 1.062 mpn *L. pneumophila*/100 mL was obtained for Legiolert. In the summer months, the concentration of *Legionella* detected by ISO increased and reached its maximum in July with 3.700 cfu/100 mL. The proportion of *L. pneumophila* detected with Legiolert is about 10 %. At the investigation in August, legionellae were not detected with both methods. In September, the *Legionella* concentration increased to the mid-hundred range with both methods. While the concentration of *L. pneumophila* dropped to a quarter and remains the same to the end of the observation (Legiolert curve), the concentration of non-*pneumophila Legionella* detected by ISO continued to rose until November.

On the stainless steel plates of the short interval, *Legionella* was frequently but not always detected. Mostly concentrations in the hundreds were found. In September, the highest concentration of 1,500 cfu/25 cm^2 was determined. On the stainless steel plates of the long-term

interval, continuously increasing concentrations were recorded until the beginning of September. Afterwards the concentrations fell continuously until the end of the observation period. On the polyethylene plates of the short interval, concentrations in the range of hundreds were consistently detected over the entire observation period. Here, too, the highest concentration was recorded in September. The concentrations on the polyethylene plates of the long period of observation varied slightly. In February and at the end of September no Legionellae were detected.

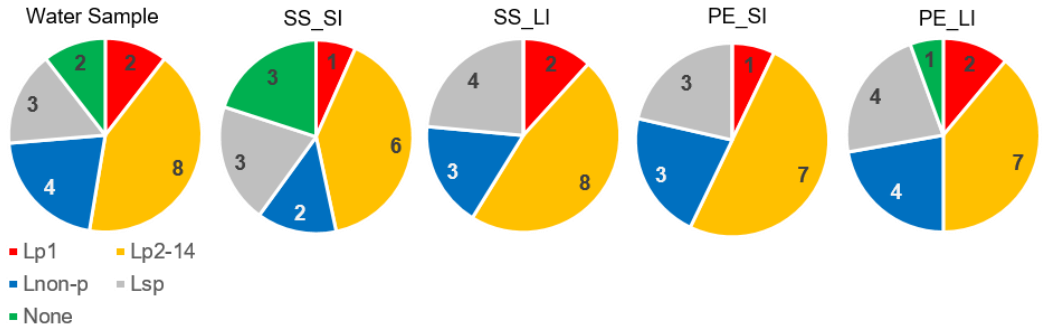


Figure 48: CT3 - Distribution of *Legionella* strains in water samples (ISO method; n = 11) and on the different short-term (n = 10) and long-term (n = 9) plates.
SS: stainless steel; PE: polyethylene; xx-LI: long interval plate; xx_SI: short interval.

The distribution of *Legionella* strains in the water samples and on the plates is shown in Figure 48. *L. pneumophila* from serogroups 2 - 14 was predominantly found in Cooling Tower 3. Often, several colony types of *L. pneumophila* from the range of serogroups 2 - 14 occurred. Especially in the warmer months co-contaminations with *Legionella sp*. were observed, some of which showed a positive reaction in the latex test ("*L*. non-*pneumophila*"). Table 42 helps to trace when which co-contaminations occurred.

Table 42: CT3 - Distribution of Legionella in water samples (ISO method) and on the different plates.
SS: stainless steel; PE: polyethylene; xx-LI: long interval plate; xx_SI: short interval.

Date of investigation	Water				SS_SI				SS_LI				PE_SI				PE_LI			
	Lp1	Lp2-14	Lnon-p	Lsp	Lp1	Lp2-14	Lnon-p	Lsp	Lp1	Lp2-14	Lnon-p	Lsp	Lp1	Lp2-14	Lnon-p	Lsp	Lp1	Lp2-14	Lnon-p	Lsp
21-Feb		x			-				-				-				-			
21-Mar		x							-					x			-			
18-Apr								x				x				x	x	x		
15-May		x							x	x				x				x		
12-Jun			x			x				x				x			x	x		
15-Jul	x	x	x		x	x			x	x			x	x					x	x
7-Aug	x	x		x						x	x	x		x				x	x	x
4-Sep						x	x	x		x	x	x			x	x		x	x	x
30-Sep		x	x	x		x		x		x		x			x	x				
28-Oct		x		x		x				x				x				x		
25-Nov		x	x			x	x			x	x			x	x			x	x	x

Since legionellae were detected in the majority of the biofilm smears of the plates from Cooling Tower 3, a statistical data pair comparison was carried out according to the laboratory's internal "0.5 log rule". Comparing the short-time plates, one value was the same for both plate types, four results of the stainless steel plates were higher and five results of the polyethylene plates were higher. Of the four higher stainless steel results, two were significantly higher according to the internal laboratory rule. Of the five higher polyethylene results, four were significantly higher according to the laboratory rule. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly. Comparing the long-term plates, one value was also the same for both plate types, six results of the stainless steel plates were higher and two results of the polyethylene plates were higher. Of the six higher stainless steel results, five were significantly higher according to the internal laboratory rule. Of the two higher polyethylene results, one was significantly higher according to the laboratory rule. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly. Looking at the short and long-term stainless steel plates, all nine values of the long interval were significantly higher according to the laboratory rule. The performance of the Mann-Whitney-U-test indicated that these data sets differed significantly. Regarding the short and long-term polyethylene plates, two data pairs showed the same value, two results of the short interval were higher and five of the long interval. According to the laboratory rule, these higher results were significantly higher. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly.

The two *P. aeruginosa*-curves, based on ISO and Pseudalert results from the water samples, followed a similar pattern until testing in May. First, the curves fell slightly to about 100 cfu or mpn/100 mL and then increased to the lower 10^3 cfu or mpn/100 mL range. The ISO method provided higher results from February to May. While a stagnation in the range of 10^3 mpn/100 mL was observed for *P. aeruginosa* concentrations determined with Pseudalert over the summer months until August, the concentrations determined with the ISO method fluctuated strongly. In June, *P. aeruginosa* was not detected with the ISO method, but in July, concentrations of 1,300 cfu/100 mL were recorded, followed by a decrease and absence of detection with the ISO method until the end of the observation period. After the stagnation period of *P. aeruginosa* detected with Pseudalert in the summer months, early September *P. aeruginosa* was not detected with this method. Until sampling at the end of September, the concentration increased by almost four $\log_{10}$ levels and decreased by one $\log_{10}$ level in each of the last two investigations.

The growth of *P. aeruginosa* on the long-term plates is almost congruent for the two plate types. By May, the concentration increased to 10^3 cfu/25 cm^2 and stagnated from this point until the end of the investigation period with slight fluctuations and an increase of almost one log

level at the end of September. *P. aeruginosa* was not detected on the short-term stainless steel plates in March and April. Between May and July, concentrations between 10^2 and 10^3 cfu/25 cm^2 were detected. From August onwards, concentrations in the range of 10^3 to more than 10^4 cfu/25 cm^2 were found on the stainless steel plates.

P. aeruginosa was not detected on the polyethylene plates in April and early September. During the remaining months, the microorganism was determined in varying concentrations ranging from 45 cfu to 10^4 cfu/25 cm^2.

Since *P. aeruginosa* was detected in the majority of the biofilm smears of the plates from Cooling Tower 3, a data pair comparison is carried out here according to the laboratory's internal "0.5 log rule". For the ten examined plate pairs of the short interval, one data pair delivered the same value for both plate types. Six results were higher from the stainless steel plates and three from the polyethylene plates. Three results were significantly higher for each plate type according to the laboratory's internal rule. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly. For the plate pairs of the long interval, the same value on both plate types was recorded one time. Four results each were higher on the stainless steel and the polyethylene plates, but according to the internal laboratory rule, only one stainless steel-result was significantly higher. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly. Comparing the stainless steel plates, five results of the short interval were higher, two of them significantly. Four results of the long interval plates were higher, also two of them significantly according to the laboratory rule. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly. Regarding the polyethylene plates, two results of the short interval were significantly higher. Seven results of the long-term plates were higher, four of them significantly according to the laboratory rule. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly.

Initially it was planned to determine the biofilm mass on the last long-term plates after the ten-month observation period for all cooling towers. In Cooling Towers 1, 2 and 4 so little biomass was obtained from 100 cm^2 that the tare value of the Petri dish weighed on the day before sampling was more than the Petri dish plus biomass weighed the next day after sampling.

On the long-term stainless steel plate removed on the last sampling date, a wet weight of 10.224 g/100 cm^2 and a dry matter of 1.304 g/100 cm^2 was obtained. On the long-term polyethylene plate, a wet weight of 6.143 g/100 cm^2 and dry matter of 0.716 g/100 cm^2 were determined. The proportions of the dry matters from the wet weights amounted to 12,75 % for the stainless steel plate and 11.67 % for the polyethylene plate.

3.3.2.4 Cooling Tower 4

Cooling Tower 4 was characterized by visually clean plates as shown in Figure 49. The biocide treatment was performed with chlorine and sodium bromide depending on the redox potential. During removing and swabbing off the plates, only a very thin layer of a soft biofilm on both the plates of the short and the long observation interval was visible over the entire investigation period.

Figure 49: Plates after removal from Cooling Tower 4.
a) stainless steel plate and b) polyethylene plate from the short interval (four weeks), c) stainless steel and d) polyethylene plate from the long interval (28 weeks).

In Cooling Tower 4, the HPC-22 °C-curve fluctuated between February and May between 60 cfu and 9,500 cfu/mL. The HPC-36 °C-curve dropped continuously from 9,500 cfu to 140 cfu/mL in May. Between June and August, the HPC-36 °C-concentration remainded stable within in the range between 10^2 and 10^3 cfu/mL, then rose by the factor ten and stagnated with a slightly decreasing tendency until the end of the monitoring. The HPC-22 °C-results varied

within a $\log_{10}$ level from June to the end of the observation period with a slightly increasing trend until the end of September and then with a slightly decreasing trend. HPC at 22 °C incubation temperature were higher in three of eleven water samples compared to HPC at 36 °C. The four special biocide dosages apparently provided no strong reducing effect on HPC in the water samples.

On the polyethylene plates of the long observation interval, the highest concentrations were obtained for HPC at 22 and 36 °C incubation temperature in the first examination after eight weeks of exposure in the cooling tower. In the following months, a decrease of HPC at both incubation temperatures was observed. Between July and early September, the growth of heterotrophic bacteria on the polyethylene plates stagnated and increased slightly until the end of the investigation period. Even on the stainless steel plates of the long-term interval, higher concentrations of HPC at 22 and 36 °C were detected in the first smears than in the following ones. After the decrease, a constant slight increase in HPC was observed over the entire period of investigation. Especially in the April and May investigations, HPC were clearly higher on the polyethylene plates, whereas the counts in the other investigations settled at the same level. For both plate materials and incubation temperatures, the HPC graphs of the long-term plate smears tend to run parallel to the HPC in the water samples.

Fluctuating HPC were observed on both plate types of the short interval at both incubation temperatures. The profiles of HPC grown on the two different materials differed when the same incubation temperature was observed. However, the HPC profiles of the same plate material were similar for the two incubation temperatures. On the short-term stainless steel plates, minima were recorded for both incubation temperatures in March, June, August and November. On the short-term polyethylene plates, minima were observed for both incubation temperatures in June, early September and November. Maxima were recorded for both plate types and incubation temperatures in April, July and October.

For the majority of colonies grown on the Yeast extract agar plates, the same colony morphology types were observed for the water samples, the short-term and the long-term plates of both materials. Detected colonies were not further identified.

In order to assess whether the HPC of the different plate types and test intervals differed significantly or could be considered as the same, the laboratory "0.5 log level-rule" was applied.

Comparing the HPC at 22 °C incubation temperature grown on the short-interval plates, five results of each the stainless steel and polyethylene plates were higher than the corresponding result of the other plate type. Using the laboratory's own "0.5 log level-rule" it was determined that three results for HPC at 22 °C from the stainless steel and five results from the polyethylene plates were significantly higher compared to the corresponding result of the other plate

type. Hence, the results differed less than a half log level in two investigations. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Looking at the long-term plates for HPC at 22 °C, in absolute numbers, three results of the stainless steel plates were higher and six results from the polyethylene plates were higher. Using the internal laboratory "0.5 log level-rule", no result of the long-term stainless steel plates was significantly higher and two results of the polyethylene plates were significantly higher. Accordingly, seven results ranged within a half log level. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Comparing the results of the short-term and long-term stainess steel plates for HPC at 22 °C, six results were higher from the plates of the short-term interval and three results were higher for the long-term interval. Using the "0.5 log level-rule", four results of the short-term plates and three of the long-term plates were significantly higher. Two results can be considered as equally according to the “0.5 log level-rule”. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly. For the polyethylene plates, three results were higher from the plates of the short-term interval and six results were higher for the long-term interval. Using the "0.5 log level-rule", two results of the short interval and four results of the long interval were significantly higher. Accordingly, the results of three removal dates can be regarded as equally. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly.

Regarding the HPC at 36 °C grown on the short-term plates, seven results from the stainless steel plates and three counts of the polyethylene were higher. Applying the laboratory “0.5 log level-rule” six results from the stainless steel plates were significantly higher compared to the corresponding short-term interval polyethylene steel plate results. Two counts of the polyethylene plates were significantly higher. Two results from the two types of short-time plates differed less than a half log level. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Comparing the HPC at 36 °C from the long-term plates, of the three higher counts on the stainless steel plates, one result was significantly higher. Of the five higher results from the polyethylene plates, four were significantly higher according to the "0.5 log level-rule". Accordingly, four samples differed less than a half log level. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Table 43: Cooling Tower 4 - Microbial concentrations in water samples.
UQL: upper quantification level. In brackets and in grey the 1 mL direct plating results are shown.

Examination Date	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	*Legionella* spp. ISO [cfu/100 mL]	*L. pneumophila* IDEXX [mpn/100 mL]	*P. aeruginosa* ISO [cfu/100 mL]	*P. aeruginosa* IDEXX [mpn/100 mL]
21.02.2019	9,500	9,500	635	1,244	2,700	$1{,}4*10^4$
21.03.2019	780	570	1,300	1,546	>UQL	$1.7*10^4$
18.04.2019	7,600	520	2,000	1,867	6,500	1,100
15.05.2019	60	140	3,300	8,540	2.420 (1,300)	1,664
12.06.2019	530	590	640	896	>UQL (11,400)	4,352
15.07.2019	230	730	300	386	130 (<DL)	41
07.08.2019	560	120	$2.4*10^4$	$3.2*10^4$	1,700 (<DL)	2,400
04.09.2019	760	2,610	$6.5*10^4$	$9.4*10^4$	n (2,900)	8,664
30.09.2019	1,280	4,380	$1.7*10^4$	$2.8*10^4$	>UQL (3,800)	2,755
28.10.2019	450	3,700	1,200	3,100	>UQL (1,500)	1,086
25.11.2019	240	2,430	5,600	$1.6*10^4$	>UQL (700)	305

Table 44: Cooling Tower 4 - Microbial concentrations in the biofilms grown on a) stainless steel and b) polyethylene plates.
DL: detection limit.

a)

[cfu/25 cm²]	Short interval observation				Long interval observation			
	HPC 22 °C	HPC 36 °C	*Legionella* spp.	*P. aeruginosa*	HPC 22 °C	HPC 36 °C	*Legionella* spp.	*P. aeruginosa*
21.03.2019	100	<DL	<DL	<DL				
18.04.2019	$3.3*10^5$	$4.3*10^5$	200	180	$9.8*10^4$	$1.1*10^5$	70	260
15.05.2019	$2.0*10^4$	$1.4*10^4$	100	<DL	6,400	$8.8*10^4$	65	70
12.06.2019	600	2,300	25	15	$1.4*10^4$	6,700	60	170
15.07.2019	$7.3*10^5$	$6.6*10^5$	2.400	300	4,400	$1.3*10^4$	5	60
07.08.2019	800	$3.2*10^4$	<DL	45	$1.2*10^4$	$5.2*10^4$	130	<DL
04.09.2019	$1.6*10^5$	$2.2*10^5$	945	$2.2*10^4$	$2.1*10^4$	$1.1*10^5$	1,790	20
30.09.2019	$1.0*10^5$	$2.0*10^5$	675	50	$6.4*10^4$	$1.4*10^5$	1,800	5
28.10.2019	$1.2*10^6$	$9.7*10^5$	180	$2.6*10^5$	$1.4*10^5$	$2.2*10^5$	505	65
25.11.2019	7,600	7,200	370	5	$1.2*10^5$	$3.8*10^5$	600	15
b)								
21.03.2019	600	700	<DL	40				
18.04.2019	$3.6*10^5$	$6.8*10^5$	370	100	$9.2*10^6$	$1{,}7*10^7$	2,500	315
15.05.2019	$8.6*10^4$	$1.8*10^5$	75	<DL	$5.8*10^5$	$1.1*10^6$	385	$1.0*10^4$
12.06.2019	3,200	3,300	55	55	$2.3*10^4$	$3.0*10^4$	195	235
15.07.2019	$1.7*10^4$	$4.9*10^4$	40	60	4,300	$6.0*10^4$	120	15
07.08.2019	7,400	$3.6*10^4$	345	55	$2.7*10^4$	$3.0*10^4$	160	15
04.09.2019	300	400	150	<DL	8,100	$1.9*10^4$	1,000	<DL
30.09.2019	$1.3*10^4$	$3.6*10^4$	675	275	$1.1*10^5$	$1.8*10^5$	1,800	45
28.10.2019	$7.6*10^5$	$8.2*10^5$	70	$1.4*10^5$	$2.0*10^5$	$2.2*10^5$	250	455
25.11.2019	$3.2*10^4$	$6.2*10^4$	875	15	$6.0*10^4$	$1.7*10^5$	2,100	60

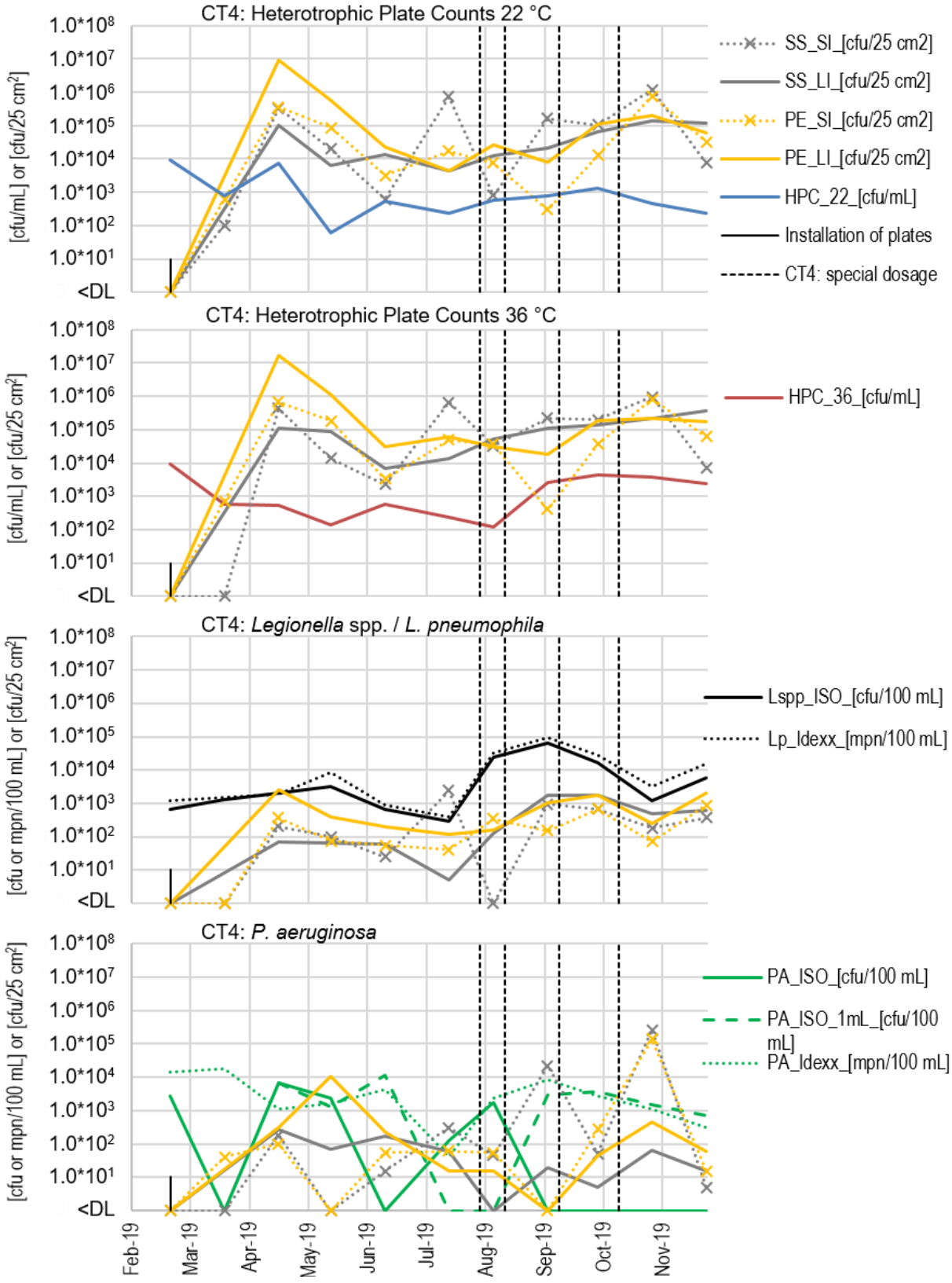


Figure 50: CT4 - Microbial growth on the SS and PE plates and in the water sample.
SS: stainless steel; PE: polyethylene; xx-LI: long interval plate; xx_SI: short interval.

Considering the results for HPC at 36 °C grown on the stainless steel plates of the short and long observation intervals, five results were higher from the short interval plates and four from the long interval plates. Three results were significantly higher for the short interval and two results from the long-term plates were significantly higher according to the "0.5 log level-rule". Results from four plate investigations differed by less than a half log level. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly. Regarding the short-term and long-term polyethylene plates one result from the short interval was higher and eight from the long interval were higher. No result of the short interval, but seven results of the long interval were significantly higher compared to the result of the short-interval from the same removal date. Hence, two results ranged within a half log level. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly.

As mentioned above, HPC at 22 °C incubation temperature were higher in three of eleven water samples compared to HPC at 36 °C. HPC at 36 °C were higher in six samples. In two samples, the count was the same for 22 and 36 °C. Applying the “0.5 log level-rule” two results of the HPC at 22 °C were significantly higher than the count for 36 °C and four results were significantly for HPC at 36 °C. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

The legionellae curves of Legiolert and ISO results are very similar. Legiolert provided the higher result in ten samples. Both curves showed a slightly increasing trend until the investigation in May. During this period, concentrations between 635 cfu and 3,300 cfu/100 mL were determined with ISO, and between 1,240 mpn and 8,500 mpn/100 mL with Legiolert. From May onwards, both curves fell to 300 cfu/100 mL (ISO) and 386 mpn/100 mL (Legiolert) by July. From August onwards, the concentrations increased by two log levels and reached peak values of 65,000 cfu/100 mL (ISO) and almost 94,000 mpn/100 mL (Legiolert). After the first two peracetic acid shock dosages on July 31st and August 13th, the legionellae concentrations consequently continued to rise. After the shock dosages on September 10th and October 11th, decreasing legionellae concentrations were observed. In the November investigation, a renewed increase in concentrations was recorded. The concentration determined with Legiolert was at 15,700 mpn/100 mL three times higher than the ISO result (5,600 cfu/100 mL).

Legionellae were detected in almost all biofilm smears of the plates. The profiles of both plate types of the long interval tended to be parallel to the graphs of the water sample. Except for the peak of the polyethylene plate in April, lower *Legionella* concentrations were observed on the long interval plates in the first half of the observation period with a decrease between June and July and higher concentrations in the second half of the observation period. Legionellae

were not found on the short-term plates on both materials in March. Subsequently, concentrations between 10 cfu and 1,000 cfu/25 cm^2 were always detected with increasing tendency over the entire observation period.

The distribution of *Legionella* strains in the water samples and on the plates is shown in Figure 51. *L. pneumophila* from serogroups 2 - 14 was predominantly found in Cooling Tower 4. Often, several colony types of *L. pneumophila* from serogroup 1 and the range of serogroups 2 - 14 occurred together. Sporadic co-contaminations with *Legionella species* were observed, some of which showed a positive reaction in the latex test ("*L.* non-*pneumophila*"; abbreviated "Lnon-p"). Table 45 helps to trace when which co-contamination occurred.

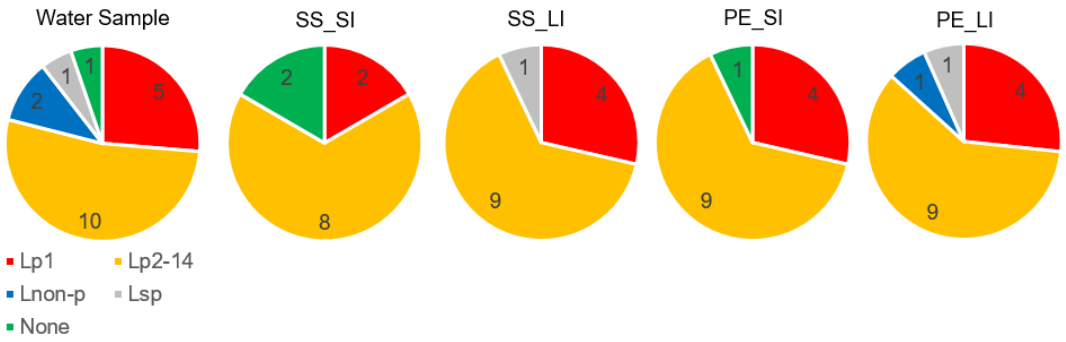


Figure 51: CT4 - Distribution of *Legionella* strains in water samples (ISO method; n = 11) and on the different short-term (n = 10) and long-term (n = 9) plates.
SS: stainless steel; PE: polyethylene; xx-LI: long interval plate; xx_SI: short interval.

Table 45: CT4 - Distribution of *Legionella* in water samples (ISO method) and on the different plates.

Date of investigation	Water				SS_SI				SS_LI				PE_SI				PE_LI			
	Lp1	Lp2-14	Lnon-p	Lsp	Lp1	Lp2-14	Lnon-p	Lsp	Lp1	Lp2-14	Lnon-p	Lsp	Lp1	Lp2-14	Lnon-p	Lsp	Lp1	Lp2-14	Lnon-p	Lsp
21-Feb	x	x			-				-				-				-			
21-Mar	x	x							-								-			
18-Apr	x	x				x			x	x				x			x	x		
15-May	x	x			x	x			x	x			x	x			x	x		
12-Jun		x				x				x			x	x				x		
15-Jul	x	x	x		x	x			x	x			x	x			x	x		
7-Aug									x	x		x	x	x			x	x		
4-Sep		x				x				x				x				x		
30-Sep		x				x				x				x				x		
28-Oct		x				x				x				x				x		
25-Nov		x	x	x		x				x				x				x	x	x

Since *Legionella* spp. was detected in the majority of the biofilm smears of the plates in Cooling Tower 4, a data pair comparison is carried out here according to the laboratory's internal "0.5 log rule". Comparing the short-time plates two values were the same for both plate types, four results of each plate type were higher. Of the four higher stainless steel results, two were significantly higher according to the internal laboratory rule. Of the four higher polyethylene

results, one was significantly higher according to the laboratory rule. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Looking at the long-term plates, one value was the same for both plate types, two results of the stainless steel plates were higher and six results of the polyethylene plates were higher. Of the two higher stainless steel results, no one was significantly higher according to the internal laboratory rule. Of the six higher polyethylene results, five were significantly higher according to the laboratory rule. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Regarding the short and long-term stainless steel plates, three values of the short interval and six values of the long interval were higher. According to the laboratory rule for each observation interval one result was significantly higher. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly. Comparing the short and long-term polyethylene plates, one result of the short interval was higher and eight of the long interval. Five results of the long interval were significantly higher according to the laboratory rule. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly.

The curve for *P. aeruginosa* detected by the ISO method (10 mL membrane filtration) seemed to fluctuate strongly by regarding the chart in Figure 50. In fact, the concentration was not determinable in June and from September to November due to exceedance of the upper quantification limit of 2,000 cfu/100 mL. The count of 6,500 cfu/100 mL derived from 1 mL sample volume that was inadvertently tested by both methods instead of 10 mL. The 1 mL sample volume results are shown in brackets in Table 43 in the column "*P. aeruginosa* ISO [cfu/100 mL]" next to the 10 mL-results of the ISO method. The results were included in the comparative analysis in section 3.3.5.2 although the procedure does not correspond to the requirements of the ISO method. Concentrations for *P. aeruginosa* could be quantified due to the smaller sample volume and a similar profile to that of the Pseudalert curve was observed. With Pseudalert, *P. aeruginosa* was detected over the entire observation period. When the monitoring started in February, 14,000 mpn/100 mL were recorded. With the sampling in March, a slight increase was observed. Until the April investigation, the concentration decreased by one $\log_{10}$ level and then increased slightly until June. In the July investigation, a reduction of the concentration by two $\log_{10}$ levels was observed. The next peak was recorded at the beginning of September, in which about 8,700 mpn/100 mL were detected. Subsequently, a decrease of the curve to 300 mpn/100 mL was noticed until the end of the investigation period.

The growth of *P. aeruginosa* on the plates was also characterized by fluctuations. On both types of short-term plates, concentrations were found usually in the lower hundreds. Much

higher concentrations were found on two short-term stainless steel plates in early September and October. This was also the case for the October polyethylene short-term plate. On the stainless steel plates of the long interval, varying concentrations between 5 cfu and 260 cfu/25 cm^2 were found. On the polyethylene long-term plates, fluctuating concentrations between 15 cfu and 455 cfu/25 cm^2 and in May a significantly higher concentration were detected. No parallelism between short-term and long-term plates or the concentration in the water sample was identified.

Since *P. aeruginosa* was detected in the majority of the biofilm smears of the plates from Cooling Tower 4, a data pair comparison is carried out here according to the laboratory's internal "0.5 log rule". Comparing the short-time plates, one value was the same for both plate types, four results of the stainless steel plates and five results of the polyethylene plates were higher. Of the four higher stainless steel results, two were significantly higher according to the internal laboratory rule. Of the five higher polyethylene results, three were significantly higher according to the laboratory rule. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Looking at the long-term plates, two results of the stainless steel plates and seven results of the polyethylene plates were higher. The two higher stainless steel results were significantly higher according to the internal laboratory rule. Of the seven higher polyethylene results, five were significantly higher according to the laboratory rule. The performance of the Wilcoxon signed-rank test indicated that these data sets did not differ significantly.

Regarding the short and long-term stainless steel plates, five values of the short interval and four values of the long interval were higher. According to the laboratory rule five results of the short interval and two of the long interval were significantly higher. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly. Comparing the short and long-term polyethylene plates, one result was equal for both intervals and four results were higher for each interval. The four results of the short interval and three results of the long interval were significantly higher according to the laboratory rule. The performance of the Mann-Whitney-U-test indicated that these data sets did not differ significantly.

3.3.3 Microbiological composition of the water samples from the four cooling towers

The development of the microbiological concentrations in the water samples from the four cooling towers over the ten-month observation period is presented in Figure 52 and the characteristics of the cooling towers, similarities or differences are highlighted. Figure 53 visualizes the value ranges of the individual parameters for the four cooling towers via box plots. Although the data sets cover different sampling periods, they are presented side by side in boxplots. In this way, the concentration differences of the two sampling intervals are easily illustrated.

3.3.3.1 Microbial concentrations in the water samples of the four cooling towers

Regarding the four charts in Figure 52, the curves of the Cooling Towers form characteristic, clearly assignable profiles. Cooling Tower 1 was characterized by low and tending uniform HPC curves, except for the period in which no biocide dosing took place. *Legionella* and *P. aeruginosa* curves were characterized by on-off detection at the lower quantification level. The ISO and IDEXX curves scarcely differed. The profile of Cooling Tower 2 was characterized by jagged curves. Legionellae and *P. aeruginosa* were monthly alternating absent or detected in low concentrations. The fluctuations of HPC are characteristic for Cooling Tower 2. The profile of Cooling Tower 3 is characterized by fluctuating HPC and *Legionella* curves with maxima in July and end of September. The profile of Cooling Tower 4 is characterized by the continuous *Legionella* detection and the highest *Legionella* concentrations as well as the regularly running HPC curves. Due to the frequent results above the upper quantification limit in the detection of *P. aeruginosa* in the water samples from Cooling Tower 4, 1 mL sample volume was additionally tested (results taken from 1 mL volume are traceable in Table 43). The maximum result from 1 and 10 mL sample volume was taken for further analyses.

In the charts of Cooling Towers 2, 3 and 4, a parallelism of the *Legionella* and HPC curves was observed. In general, HPC and *Legionella* do not correlate (8, 27). Thus, no further correlation analyses of these parameters were performed.

The concentration ranges of the different microbiological parameters in the water samples are shown for the four Cooling Towers as box plots in Figure 53. The corresponding concentrations of the microbiological parameters are listed in Table 46. In the boxplots, the upper and lower ends represent the minima and maxima. The upper edge of the box reflects the 75 % quartile (Q3), the horizontal line in the box the median and the lower edge of the box the 25 % quartile (Q1). The less the boxplots overlap, the more the data sets differ. It should be noted that the HPCs were referred to one millilitre according to the 42nd BImSchV, while *Legionella* and *P. aeruginosa* were related to 100 mL.

For **HPC at 22 °C**, Cooling Tower 1 provided on the one hand the lowest interquartile range, in which 50 % of the results were recorded (145 cfu to 550 cfu/mL, median at 290 cfu/mL), and on the other hand the widest range of values and the highest of all HPC 22 °C results with 305,000 cfu/mL. This high count was obtained when the biocide dosage was deactivated. For Cooling Tower 2, the largest range between lower and upper quartile (190 cfu to 1,380 cfu/mL, median at 650 cfu/mL) and the second highest maximum value of 71,900 cfu/mL were recorded. Of the four cooling towers, Cooling Tower 2 provided the highest median and upper quartile. Only in Cooling Tower 2, HPC at 22 °C were not detected in one sample. For Cooling Tower 3, the range of values extended from 20 cfu to 18,800 cfu/mL, with the lower quartile at 265 cfu/mL, the median at 530 cfu/mL and the upper quartile at 1,380 cfu/mL. The HPC 22 °C values of Cooling Tower 4 covered the smallest range from 60 cfu to 9,500 cfu/mL, with the lower quartile at 345 cfu/mL, the median at 560 cfu/mL and the upper quartile at 1,030 cfu/mL. All data sets of HPC at 22 °C of the four cooling towers were not normally distributed. The Mann-Whitney U test (two-tailed hypothesis, significance level at 5 %) was used to check whether the data sets differed significantly or not. The test indicated that the data sets did not differ significantly ($p > 0.05$).

Cooling Tower 1 also provided the widest range of values for **HPC at 36 °C** (below the detection limit up to 71,900 cfu/mL) and the lowest range between the lower and upper quartiles (lower quartile at 45 cfu/mL, median at 90 cfu/mL, upper quartile at 140 cfu/mL). Only in Cooling Tower 1, HPC at 36 °C were not detected in one sample. The value range of Cooling Tower 2 extended from 10 cfu to 71,900 cfu/mL, with the lower quartile at 70 cfu/mL, the median at 330 cfu/mL and the upper quartile at 2,035 cfu/mL. Due to the log-transformed scale, the interquartile range of Cooling Tower 2 looks the broadest, but in fact, it is the range of Cooling Tower 3. The range of values of Cooling Tower 3 extended from 20 cfu to 12,800 cfu/mL, with the lower quartile at 290 cfu/mL, the median at 530 cfu/mL and the upper quartile at 4,825 cfu/mL. The range of values of Cooling Tower 4 extended from 120 cfu to 9,500 cfu/mL, with the lower quartile at 375 cfu/mL, the median at 590 cfu/mL and the upper quartile at 3,155 cfu/mL. The median of Cooling Tower 4 was the highest of the four cooling towers considering HPC at 36 °C. All data sets of HPC at 36 °C of the four Cooling Towers were not normally distributed. The data set of Cooling Tower 1 differed significantly from those of Cooling Towers 3 and 4 ($p < 0.05$; Mann-Whitney-U-test). The other data sets combinations did not differ significantly ($p > 0.05$; Mann-Whitney-U-test).

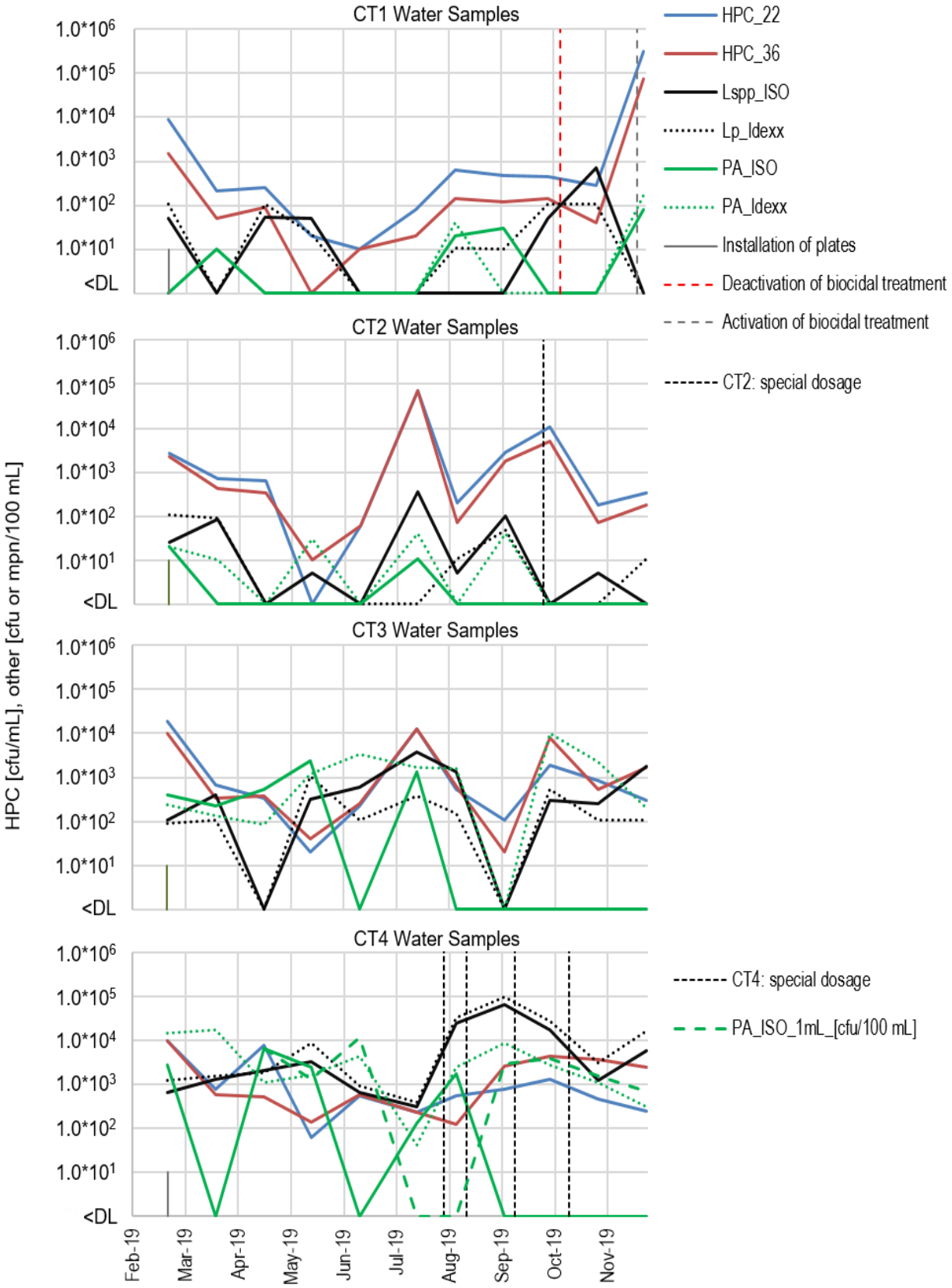


Figure 52: Microbiological composition of the water samples from the four cooling towers.

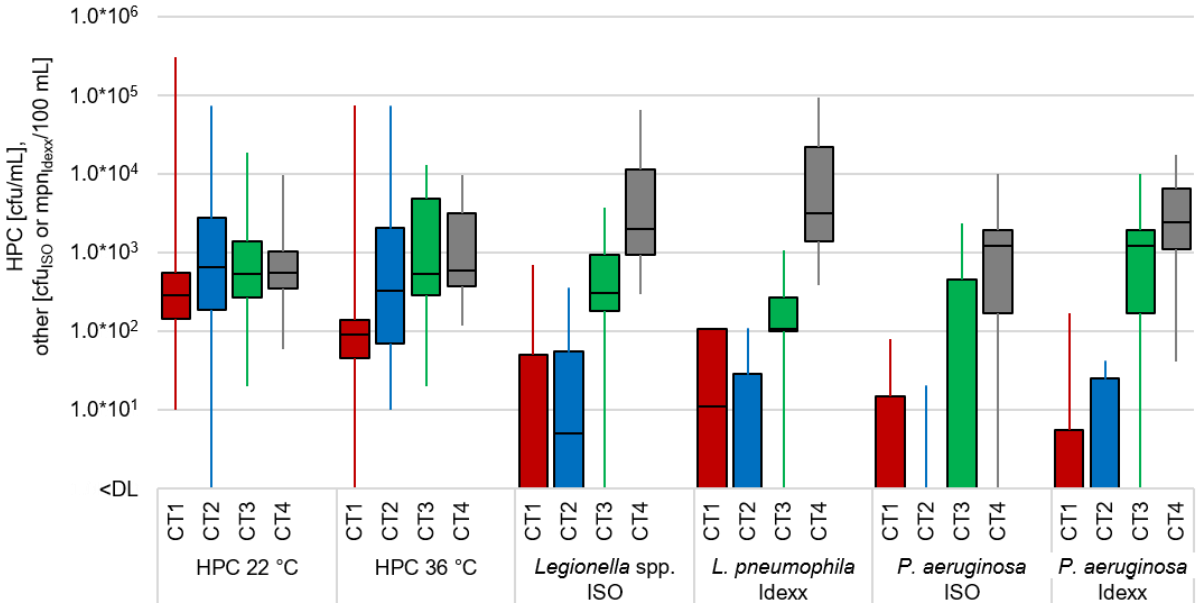


Figure 53: Value ranges of the microbiological parameters in the water samples from the four cooling towers.

Table 46: Boxplot values of the microbiological parameters in the water samples from the four cooling towers.

	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	*Legionella* spp. ISO [cfu/100 mL]	*L. pneumophila* IDEXX [mpn/100 mL]	*P. aeruginosa* IDEXX [mpn/100 mL]	*P. aeruginosa* IDEXX [mpn/100 mL]
Cooling Tower 1						
Maximum	3.1*10^5	7.3*10^4	700	108	80	171
Q3 75%	555	140	50	108	15	6
Median	290	90	<DL	11	<DL	<DL
Q1 25%	145	45	<DL	<DL	<DL	<DL
Minimum	10	<DL	<DL	<DL	<DL	<DL
Cooling Tower 2						
Maximum	7.2*10^4	7.2*10^4	350	108	20	41
Q3 75%	2,750	2,035	55	29	<DL	25
Median	650	330	5	<DL	<DL	<DL
Q1 25%	190	70	<DL	<DL	<DL	<DL
Minimum	<DL	10	<DL	<DL	<DL	<DL
Cooling Tower 3						
Maximum	1.9*10^4	1.3*10^4	3,700	1,062	2,380	9,804
Q3 75%	1,380	4,825	950	271	460	1,955
Median	530	530	310	108	<DL	1,223
Q1 25%	265	290	180	99	<DL	169
Minimum	20	20	<DL	<DL	<DL	<DL
Cooling Tower 4						
Maximum	9,500	9,500	6.5*10^4	9.4*10^4	1,1*10^4	1.7*10^4
Q3 75%	1,030	3,155	1.1*10^4	2.2*10^4	3.350	6,508
Median	560	590	2,000	3,100	2.420	2,400
Q1 25%	345	375	920	1,395	1.100	1,093
Minimum	60	120	300	386	<DL	41

For ***Legionella* spp. detected with the ISO method**, the smallest interquartile range was recorded for Cooling Tower 1. The median corresponded to the lower quartile below the detection limit. The upper quartile was 50 cfu/100 mL, the maximum was determined at the time of deactivated biocide dosing with 700 cfu/100 mL. In Cooling Tower 1, *Legionella* spp. was detected in five of the eleven samples. In total, the range of values of five legionellae containing samples from Cooling Tower 2 covered a smaller range than that of Cooling Tower 1. The lower quartile corresponded to the minimum and was below the detection limit. The median was 5 cfu/100 mL, the upper quartile 55 cfu/100 mL and the maximum 350 cfu/100 mL. The *Legionella* concentrations in Cooling Tower 3 ranged from no detection to 3,700 cfu/100 mL, with the lower quartile at 180 cfu/100 mL, the median at 310 cfu/100 mL and the upper quartile at 950 cfu/100 mL. Legionellae were determined in nine samples. In Cooling Tower 4, the highest concentrations and the broadest value range were determined. The value range extended from 300 cfu to 65,000 cfu/100 mL, with the lower quartile at 920 cfu/100 mL, the median at 2,000 cfu/100 mL and the upper quartile at 11,300 cfu/100 mL. All data sets of *Legionella* spp. tested with the ISO method of the four Cooling Towers were not normally distributed. The data set of Cooling Tower 1 did not differ significantly from that of Cooling Tower 2 ($p > 0.05$; Mann-Whitney-U-test). The data sets of Cooling Towers 1 and 2 differed significantly from those of Cooling Towers 3 and 4 ($p < 0.05$). The data set of Cooling Tower 3 differed significantly from that of Cooling Tower 4 ($p < 0.05$).

In all water samples of the four cooling towers, *L. pneumophila* strains from the range of serogroups 2 to 14 were detected most frequently. In Cooling Towers 2 and 4, *L. pneumophila* serogroup 1 strains were found second most frequently. In Cooling Towers 1 and 3, *Legionella species* strains were identified second most frequently.

For the majority, the value ranges of the ***L. pneumophila* concentrations determined with Legiolert** were very similar to those of the ISO-*Legionella* spp. concentrations of the four cooling towers. The value ranges of the Cooling Towers 1 and 2 extended from a missing *L. pneumophila* detection to 108 mpn/100 mL. In Cooling Tower 1, the lower quartile corresponded to the minimum, the upper quartile to the maximum and the median was 11 mpn/100 mL. In Cooling Tower 2, the upper quartile was 29 mpn/100 mL and the median, the lower quartile and the minimum were below the detection limit. The Legiolert-*L. pneumophila* value range of Cooling Tower 3 was smaller and lower than its ISO-*Legionella* spp. range and extended from no detection to 1,062 mpn/100 mL with the lower quartile at 99 mpn/100 mL, the median at 108 mpn/100 mL and the upper quartile at 271 mpn/100 mL. The Legiolert-*L. pneumophila* concentrations of Cooling Tower 4 were the highest of the four cooling towers and it was also higher than the ISO-*Legionella* spp. concentrations of Cooling Tower 4. The value range ex-

tended from 386 to 93,977 mpn/100 mL, with the lower quartile at 1,395 mpn/100 mL, the median at 3,100 mpn/100 mL and the upper quartile at 21,685 mpn/100 mL. All data sets of the four cooling towers for *L. pneumophila* tested by Legiolert were not normally distributed. The data set of Cooling Tower 1 did not differ significantly from that of Cooling Tower 2 ($p > 0.05$; Mann-Whitney-U-test). The data sets of Cooling Tower 1 and 2 differed significantly from those of Cooling Towers 3 and 4 ($p < 0.05$). The data set of Cooling Tower 3 differed significantly from that of Cooling Tower 4 ($p < 0.05$)Using the **ISO method *P. aeruginosa*** was detected at low concentrations in Cooling Towers 1 and 2. In Cooling Tower 1, *P. aeruginosa* was detected in four water samples, the maximum was 80 cfu/100 mL and the upper quartile 15 cfu/100 mL. Since no *P. aeruginosa* was detected in most samples, the median, lower quartile and minimum were below the detection limit. In Cooling Tower 2, *P. aeruginosa* was detected in two samples only. Thus, both quartiles, the median and the minimum were below the detection limit. The highest value was 80 cfu/100 mL. In Cooling Tower 3, *P. aeruginosa* was detected in five samples using the ISO method. The maximum was 2,380 cfu/100 mL and the upper quartile 460 cfu/100 mL. Median, lower quartile and minimum were below the detection limit. In Cooling Tower 4, *P. aeruginosa* was detected in all eleven samples. One time the count was above the upper quantification level. Afterwards 1 mL was tested additionally. The maximum amounted to 11,400 cfu/100 mL, the upper quartile 3,350 cfu/100 mL, the median 2,420 cfu/100 mL, the lower quartile 1,100 cfu/100 mL and the minimum was below the detection limit. All data sets of the four cooling towers tested for *P. aeruginosa* by the ISO method were not normally distributed. The test indicated that all data sets did not differ significantly ($p > 0.05$; Mann-Whitney-U-test).

Using **Pseudalert for the detection of *P. aeruginosa*** the value ranges of Cooling Towers 1 and 2 look very similar to those of the ISO method. In Cooling Tower 1, *P. aeruginosa* was detected in three samples. The highest value was 171 mpn/100 mL and the upper quartile 6 mpn/100 mL. Median, lower quartile and minimum were below the detection limit. In Cooling Tower 2, *P. aeruginosa* was detected in five samples. The maximum was 41 mpn/100 mL and the upper quartile 25 mpn/100 mL. Median, lower quartile and minimum were below the detection limit, too. In Cooling Tower 3, *P. aeruginosa* was detected in ten samples. The maximum was 9,804 mpn/100 mL, the upper quartile 1,955 mpn/100 mL, the median 1,223 mpn/100 mL, the lower quartile 169 mpn/100 mL and the minimum was below the detection limit. In Cooling Tower 4, *P. aeruginosa* was detected in all eleven samples. The maximum was 17,329 mpn/100 mL, the upper quartile 6,508 mpn/100 mL, the median 2,400 mpn/100 mL, the lower quartile 1,093 mpn/100 mL and the minimum 41 mpn/100 mL. All data sets of the four cooling towers tested for *P. aeruginosa* by Pseudalert were not normally distributed. The data set of Cooling Tower 1 did not differ significantly from that of Cooling Tower 2 ($p > 0.05$; Mann-Whitney-U-test). The data sets of Cooling Towers 1 and 2 differed significantly from those of

Cooling Towers 3 and 4 ($p < 0.05$). The data set of Cooling Tower 3 did not differ significantly from that of Cooling Tower 4 ($p > 0.05$).

The concentrations in the water samples for *Legionella* spp. tested by the ISO method and for *L. pneumophila* tested by Legiolert looked very similar for each of the four cooling towers. The concentrations for *P. aeruginosa* tested by the ISO method and by Pseudalert looked similar for the Cooling Towers 1 and 2, but differently for the Cooling Towers 3 and 4. The Wilcoxon signed-rank test (two-tailed consideration of the W-value with the critical value N at the significance level 0.05) was performed to get an impression if the data sets were equal or differed. In Cooling Towers 1 ,2 and 3 the ISO method data sets for *L. pneumophila* and *Legionella* spp. did not differ significantly from the Legiolert data sets, but the data sets differed significantly in Cooling Tower 4. The ISO method results for *P. aeruginosa* did not differ significantly from the Pseudalert data sets in all cooling towers. For Cooling Tower 2, the paired t-test (two-tailed hypothesis, significance level at 0.05) had to be used because the Wilcoxon signed-rank test could not be performed due to the too small data set.

At this point, it should be emphasized that the data sets in this section are actually too small for statistical analyses. Nevertheless, the statistical analyses were carried out in order to confirm the subjective impression that was gained when inspecting the data.

3.3.3.2 Exceedance of 42nd BImSchV test and action values and risk assessment

In Figure 54, the exceeded "*Legionella* test and action values of the 42nd BImSchV are shown for the different methods of this study part. The test and action values of the 42nd BImSchV are listed in Table 1. The designation "ISO_Lspp" contains all results from colony numbers of the ISO method, which were confirmed to the genus *Legionella* (*L. pneumophila* counts included). "ISO_Lp" describes colonies grown with the ISO method identified as *L. pneumophila* by the latex agglutination test. "Legiolert" includes the maximum *L. pneumophila* results of Legiolert from the both tested sample volumes.

A detailed description how the samples are evaluated depending on the method is given below.

In Cooling Tower 1, using the ISO method, *L. pneumophila* was detected exclusively in three samples below the first test value of the 42nd BImSchV. In these three samples, *L. pneumophila* was also detected by Legiolert, exceeding the test value 1 in two samples. In addition, non-*pneumophila Legionella* were determined by the ISO method in two samples, whereas the test value 1 was exceeded in one sample. In these two samples, results above test value 1 were provided by Legiolert. In two further samples, results below test value 1 were obtained by Legiolert. In total, *L. pneumophila* was detected in seven samples by Legiolert and *L. pneumophila* or a non-*pneumophila Legionella species* in five samples using the ISO method.

In Cooling Tower 2, no other *Legionella species* than *L. pneumophila* was detected in seven samples using the ISO method. Test value 1 was exceeded in one sample. By Legiolert, positive results were obtained in five samples. The test value 1 was exceeded in one sample, but this is not the same sample that exceeded test value 1 by the ISO method.

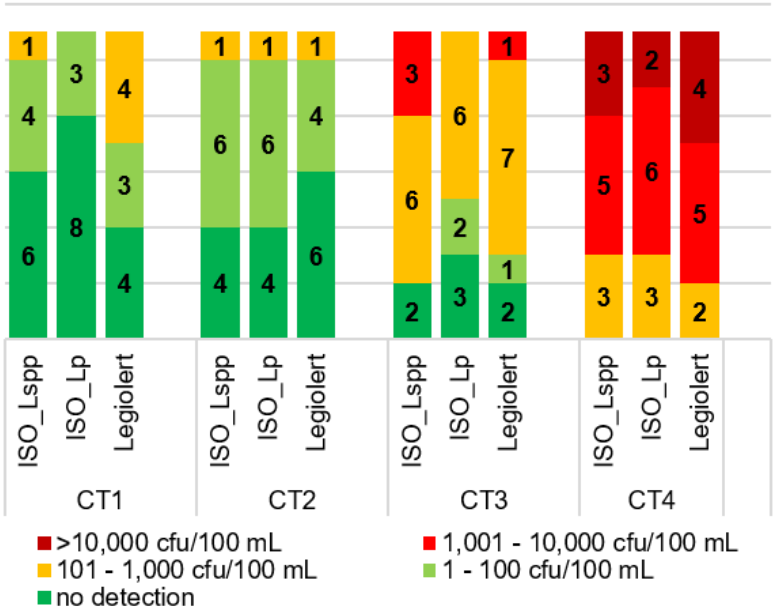


Figure 54: *Legionella* concentrations in the four cooling towers over the ten-month investigation period divided according to test and action values of the 42nd BImSchV.
The higher Legiolert result ("Legiolert max.") was applied. Dark green: below detection limit; light green: below test value 1; orange: exceedance of test value 1; light red: exceedance of test value 2; dark red: exceedance of the action value.

In Cooling Tower 3, *Legionella* was detected in nine samples by Legiolert and the ISO method. By the ISO method, four samples contained only *L. pneumophila*, one sample contained only one non-*pneumophila Legionella species* and four samples showed co-contaminations of *L. pneumophila* and at least one other *Legionella species*. In the four samples containing only *L. pneumophila* detected by the ISO method, the test value 1 was exceeded. With Legiolert the test value 1 was exceeded in these samples two times, in one sample test value 2 and in one sample no test value was exceeded. The sample that contained a non-*pneumophila Legionella species* detected by ISO, was also positive for Legiolert. The result of both methods exceeded test value 1. Three of the co-contaminated samples exceeded test value 2. When considering the *L. pneumophila* concentration determined by ISO in these samples, test value 1 is exceeded in two samples and undercut in one sample. In these samples, test value 1 was always exceeded with Legiolert.

In Cooling Tower 4, *Legionella* was detected in all samples using both methods. By the ISO method, a co-contamination of *L. pneumophila* and another *Legionella species* was detected in one sample. In the other samples, only *L. pneumophila* was detected by the ISO method. By Legiolert, two times test value 1, five times test value 2 and three times the action value

were exceeded. In one sample, the ISO result was above test value 2 and the action value was exceeded with Legiolert. In the co-contaminated sample, the *L. pneumophila* result of the ISO method exceeded test value 2, while the overall ISO result, like the Legiolert one, exceeded the action value.

In order to be able to perform a more objective risk assessment for the four cooling towers, an easy-to-apply risk factor calculation was created. The risk factor calculation presented here does not base on complicated mathematical principles as reviewed by Hamilton and Naas (57). This risk assessment bases on the exceedance of microbiological sample results according to the 42nd BImSchV *Legionella* test and action values and the HPC reference values. Risk values were assigned to the microbiological sample results. Subsequently, the risk values were averaged.

These risk values were determined by trial and error by assigning a value to each *Legionella* test and action value of the 42nd BImSchV. High concentrations should influence the risk factor calculation significantly more than low concentrations. Therefore, additional gradations were introduced in such a way that the risk values increase exponentially. Thus, when plotting the risk values against the logarithmic microbiological concentrations, an exponential function approximating the formula $y_{RF} = 0.0742x^{0.5238}$ was obtained. The 42nd BImSchV test and action values and the additional gradations function as "thresholds" for calculating the risk factor. In Figure 55, the risk factor calculation is illustrated. As mentioned above, this is an average calculation; the formula was not used for the cooling tower specific risk factor calculation.

***Legionella* spp. Thresholds [cfu/100 mL]**	**Risk Values**
≤ 100	0.5
101 - 1.000	2
1.001 - 5.000	5
5.001 - 10.000	8
10.001 - 50.000	10
> 50.000	14

HPC Thresholds [cfu/mL]	**Risk Values**
1 – 5.000	0.5
5.001 – 10.000	2
> 10.000	5

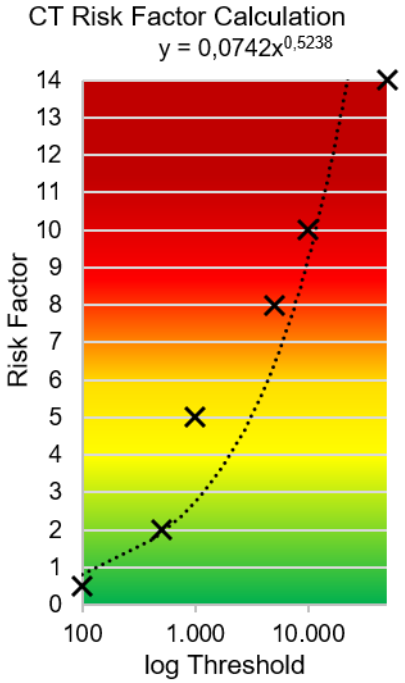
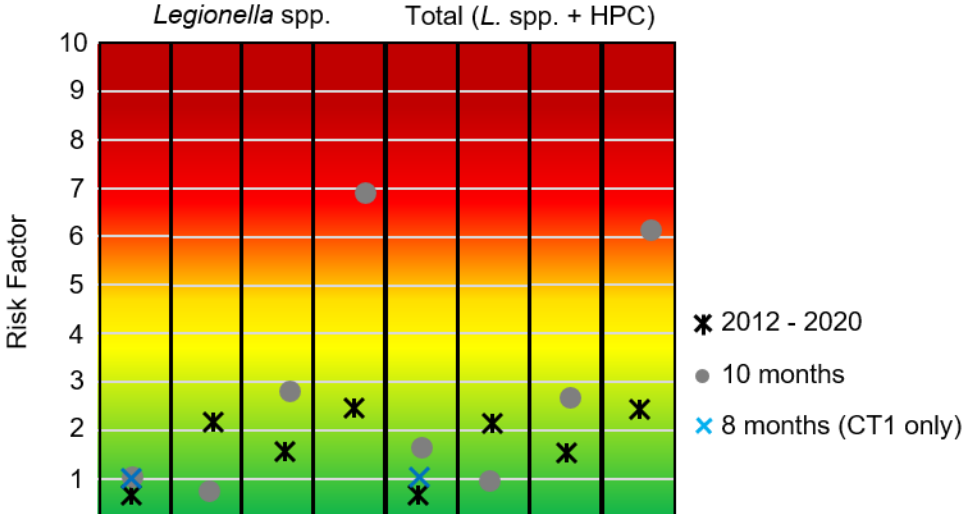


Figure 55: Cooling Tower specific risk factor calculation.

The risk value for the *Legionella* concentration of 1,000 cfu/100 mL is noticeably standing out from the trend line. This was decided because this concentration refers to test value 2 of the 42nd BImSchV, which implies measures by the operator, which should also be expressed in the risk factor calculation. In addition, cooling towers with *Legionella* concentrations higher than 1,000 cfu/100 mL have already been identified as sources of legionellosis outbreaks (164).

The cooling tower-specific *Legionella* risk factor is based on successive *Legionella* sample results. The sample results were assigned to the risk value of the highest threshold exceeded. For example, a sample result for legionella of 700 cfu/100 mL is assigned to the risk value 2. For the "total" risk factor calculation the HPC results were applied additionally. The HPC risk values were classified according to the value of 10,000 cfu/mL, which is often used as an action level (159, 172). For HPC, additional gradations were introduced as well. The focus was the cooling tower specific risk of causing legionellosis, only two HPC values were included in the risk factor calculation as described below.
The risk factor calculation is very flexible and can be designed individually. The risk factors presented in Figure 56 were calculated as follows:

For each cooling tower, a "*Legionella* spp." risk factor was calculated from the *Legionella* concentrations only (left side of Figure 56) and a "Total" risk factor including *Legionella* and HPC (right side of Figure 56).



Observation Period	CT1	CT2	CT3	CT4	CT1	CT2	CT3	CT4
2012-2020	0.7	2.2	1.6	2.5	0.7	2.1	1.5	2.4
10 months	1.0	0.8	2.8	6.9	1.7	1.0	2.7	6.2
8 months	1.0	-	-	-	1.0	-	-	-

Figure 56: Risk assessment for the four cooling towers based on different observation periods. 2012-2020: IHPH *Legionella* spp. and HPC results (IHPH calculated reference values were applied); 10 months: includes *Legionella* spp. and HPC results from the ten months observation period; the mean of each ten HPC results was calculated; 8 months: removal of CT1 *Legionella* spp. and HPC results during biocide treatment deactivation.

Two observation periods were chosen. A long-term period with IHPH data from 2012 to 2020 and the ten-month study interval including the water sample results during the biofilm experiments. To exclude the influence of biocide deactivation in Cooling Tower 1, an eight-month observation period was also calculated. The risk factors of the long-term assessment are presented as black crosses, the ones of the ten-months observation as grey circles. The risk factor of the eight-month observation excluding the influence of the deactivated biocide treatment in Cooling Tower 1 is shown as blue cross.For the "Legionella spp. risk factor, the *Legionella* concentrations of the respective observation period were assigned to the empirical risk values and all risk values were averaged. The *Legionella* spp. risk factors of the four cooling towers.

For the "total" risk factor, the *Legionella* concentrations were assigned to the risk values for the period 2012 to 2020 as described above. In addition, the IHPH reference values for HPC were included as two individual values. For the ten-month observation period, the HPC of both incubation temperatures determined over the entire period were averaged and also included in the “total” risk factor calculation as two individual values.With the help of the colour coding in the background, it is possible to quickly and easily estimate the risk potential of planktonic *Legionella* and HPC in a cooling tower.

All long-term *Legionella* and total risk factors are smaller than three. Regarding the long-term observation, Cooling Tower 1 has the lowest risk factors, followed by Cooling Towers 3 and 2. For Cooling Tower 4 the highest risk factors were recorded. In the ten-month analysis, the lowest values were calculated for Cooling Tower 2, followed by Cooling Towers 1 and 3. Cooling Tower 4 exhibited the highest risk factors. Removing the results obtained in Cooling Tower 1 under deactivated biocide treatment had no effect on the *Legionella* risk factor, but on the total risk factor.

3.3.4 Microbial composition of the biofilm grown on the stainless steel and polyethylene plates in the four cooling towers

The microbiological growth on the stainless steel and polyethylene plates from the four cooling towers over the ten-month observation period is presented in Figure 57 and Figure 58. The characteristics of the tested parameters from the biofilms grown on the different plates of the cooling towers, similarities or differences are highlighted in this section. The detailed description of the different parameters tested in the water samples and on the plates was already given for each cooling tower in section 3.3.2. In Figure 59 and Figure 60, the value ranges of the different parameters for the two plate types and examination intervals are shown as box plots for each cooling tower. The corresponding data are listed in the Table 47 and Table 48.

The charts of each cooling tower shown in Figure 57 and Figure 58 tend to be very similar for the both plate types. In each cooling tower, HPC at 22 or 36 °C were found to be the highest of all parameters for both observation intervals and plate types. In Cooling Towers 2 and 3, the HPC on both plate types of the long-term interval were usually higher than on the short-term plates. In Cooling Tower 1, it was remarkable that the HPC are often higher on the short-term plates. A mixed picture was observed in Cooling Tower 4. The short-term value generally seemed to be higher on the stainless steel plates, while on the polyethylene plates the long-term values tended to be higher. The lowest fluctuations of HPC, respectively the most uniform increase of HPC, were observed in Cooling Tower 2. In Cooling Towers 1 and 2, legionellae were detected least frequently on the stainless steel and polyethylene plates of the two observation intervals. In Cooling Towers 3 and 4, legionellae were usually detected on both plate types of both observation intervals. In general, the legionellae concentrations tended to be higher on the plates of the long interval than on the plates of the short interval.

In general, the box plots of the two plate types were similar for each cooling tower and parameter.

In both figures, the box plots of the HPC at both incubation temperatures from Cooling Tower 1 were prominent. The value ranges extended over seven $\log_{10}$ levels. Nevertheless, the range between the lower and upper quartile was the lowest compared to the other towers. On both plate types the HPC value ranges of Cooling Tower 4 were the second lowest. The HPC value ranges of Cooling Towers 2 and 3 were very similar for both plate types of the short observation period. On the long-term plates, higher concentrations were found in Cooling Tower 3 except for the HPC at 22 °C of the polyethylene plates.

Although the data sets are very small, statistical analyses were performed to get an impression of how the biofilms on the two plate types differ in the four cooling towers. Since almost all of the data sets were not normally distributed, the Mann-Whitney-U-test for unpaired samples

was performed to evaluate the relationship between the data sets. The Wilcoxon signed-rank test for paired samples was used exclusively to examine the different plate types of the same observation interval in one cooling tower. The Wilcoxon signed-rank test was partially not applicable when there were too many zero results. In this case, the paired t-test was used instead. For all statistical analyses the two-tailed hypothesis and a significance level of 5 % was applied.

Analysing the data sets of the two plate types of the same observation interval of each cooling tower with the Wilcoxon signed-rank test, the HPC grown at 22 and 36 °C did not differ significantly on the polyethylene and stainless steel plates.

Statistical analyses of the HPC data sets of one plate type for the two different investigation intervals individually for each cooling tower with the Mann-Whitney-U-test provided that the HPC results of the short- and long-term plates from Cooling Towers 1 and 4 did not differ significantly from each other, neither for 22 nor for 36 °C. In Cooling Tower 2, the HPC 22 °C data sets of the stainless steel plates did not differ significantly from each other ($p > 0.05$), but those of the polyethylene plates did ($p < 0.05$). The HPC 36 °C data sets for both plate materials of Cooling Tower 2 did not differ significantly from each other ($p > 0.05$). The HPC 22 °C data sets of the two investigation intervals of Cooling Tower 3 differed significantly from each other for both the stainless steel and the polyethylene plates ($p < 0.05$). For HPC at 36 °C only the polyethylene data sets in Cooling Tower 3 differed significantly ($p < 0.05$).

Considering the growth of HPC at 22 °C of the same plate type and observation interval of the four cooling towers, the data sets of the polyethylene plates of the short and long examination interval of Cooling Towers 1 and 2 differed significantly ($p < 0.05$). The data sets of the stainless steel and polyethylene plates of the short and long investigation interval of Cooling Towers 1 and 3 differed significantly ($p < 0.05$). The HPC 22 °C data sets of the stainless steel plate of the long interval of Cooling Towers 2 and 4 differed significantly ($p < 0.05$). The data sets of Cooling Towers 3 and 4 differed significantly in HPC at 22 °C for the polyethylene plate of the short interval and for both plate types of the long interval.

The HPC at 36 °C of all plates of the same observation interval and material in Cooling Tower 1 differed significantly from those of Cooling Towers 2 and 3. The HPC at 36 °C of the polyethylene plates of the long investigation interval of Cooling Towers 1 and 4 also differed significantly. The HPC at 36 °C of the stainless steel long-term plate differed significantly in Cooling Towers 2 and 4. The HPC 36 °C data sets of Cooling Towers 3 and 4 differed significantly for the polyethylene plates of the short interval and for both plate types of the long interval. Neither for HPC at 22 nor at 36 °C the data sets of the two observation intervals and plate materials in Cooling Towers 2 and 3 differed.

In Cooling Towers 1 and 2, legionellae were not detected frequently in the biofilm suspensions, so that no complete boxplots were generated in Figure 59 and Figure 60. On the polyethylene plates of Cooling Tower 1 and the stainless steel plates of Cooling Tower 2 of the long observation interval legionellae were not detected in the whole observation period. In contrast, legionellae were always detected on the long-term stainless steel plates from Cooling Towers 3 and 4, on the polyethylene short-time plates from Cooling Tower 3 and on the polyethylene long-term plates in Cooling Tower 4. The highest concentrations were obtained from Cooling Tower 3.

In all cooling towers, *L. pneumophila* strains were most frequently detected on the plates. On the plates of Cooling Tower 3, other *Legionella species* strains were most frequently identified in addition to *L. pneumophila.*

Analysing the data sets of the two plate types of the same observation interval of each cooling tower by the Wilcoxon signed-rank test, in all cooling towers the *Legionella* concentrations did not differ significantly on the polyethylene and stainless steel plates.

Statistical analyses by the Mann-Whitney-U-test of the *Legionella* spp. data sets of one plate type for both investigation intervals individually for each cooling tower provided that only the *Legionella* spp. concentrations of the stainless steel short- and long-term plates from Cooling Tower 3 differed significantly ($p < 0.05$).

The inter-cooling tower comparison of *Legionella* growth on the both plate types for each observation by the Mann-Whitney-U-test provided that the legionellae data sets of Cooling Towers 1 and 2 did not differ significantly ($p > 0.05$). All data sets of Cooling Towers 1 and 2 differed significantly from those of Cooling Towers 3 and 4 ($p < 0.05$). Legionellae concentrations of the stainless steel long-term plates of Cooling Towers 3 and 4 differed significantly ($p < 0.05$). The other legionellae data sets of Cooling Towers 3 and 4 did not differ significantly ($p > 0.05$).

P. aeruginosa was not detected sufficiently often in Cooling Towers 1 and 2 to create complete boxplots. The microorganism was usually detected in Cooling Towers 3 and 4 on the short- and long-term plates of both materials. For all cooling towers and both materials, the higher maximum values were recorded on the plates of the short interval. For the Cooling Towers 1, 2 and 3 the position of the *P. aeruginosa* box plots for both materials are very similar. In Cooling Tower 4, the range between the lower and upper quartiles of the stainless steel short-term plates extended over a broader range than that of the stainless steel long-term plates. Figure 60 shows that the opposite is the case for the polyethylene plates.

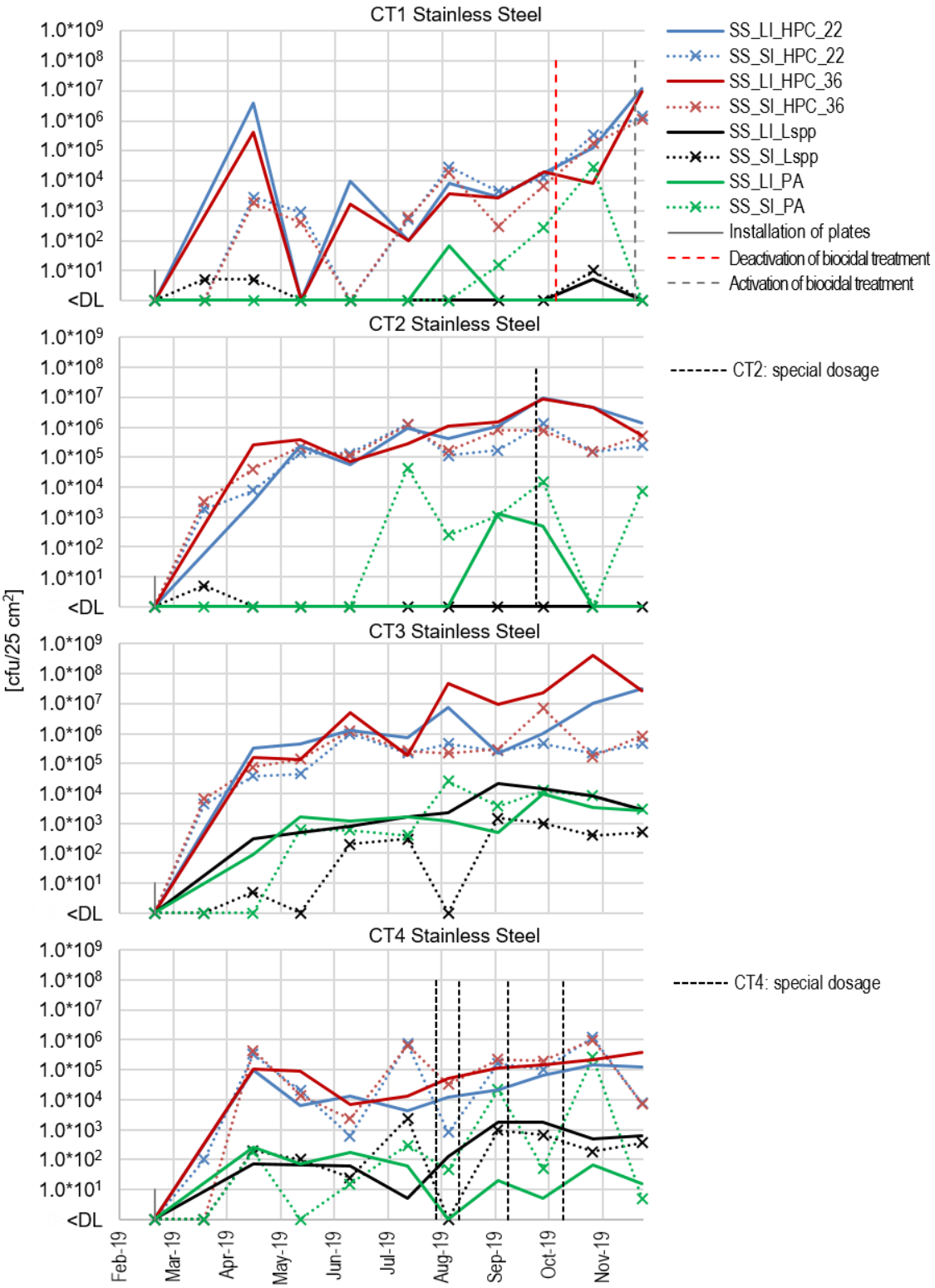


Figure 57: Microbiologicl composition of the biofilm grown on the stainless steel plates from the four cooling towers.

SS_LI: long interval stainless steel plate; SS_SI: short interval stainless steel plate.

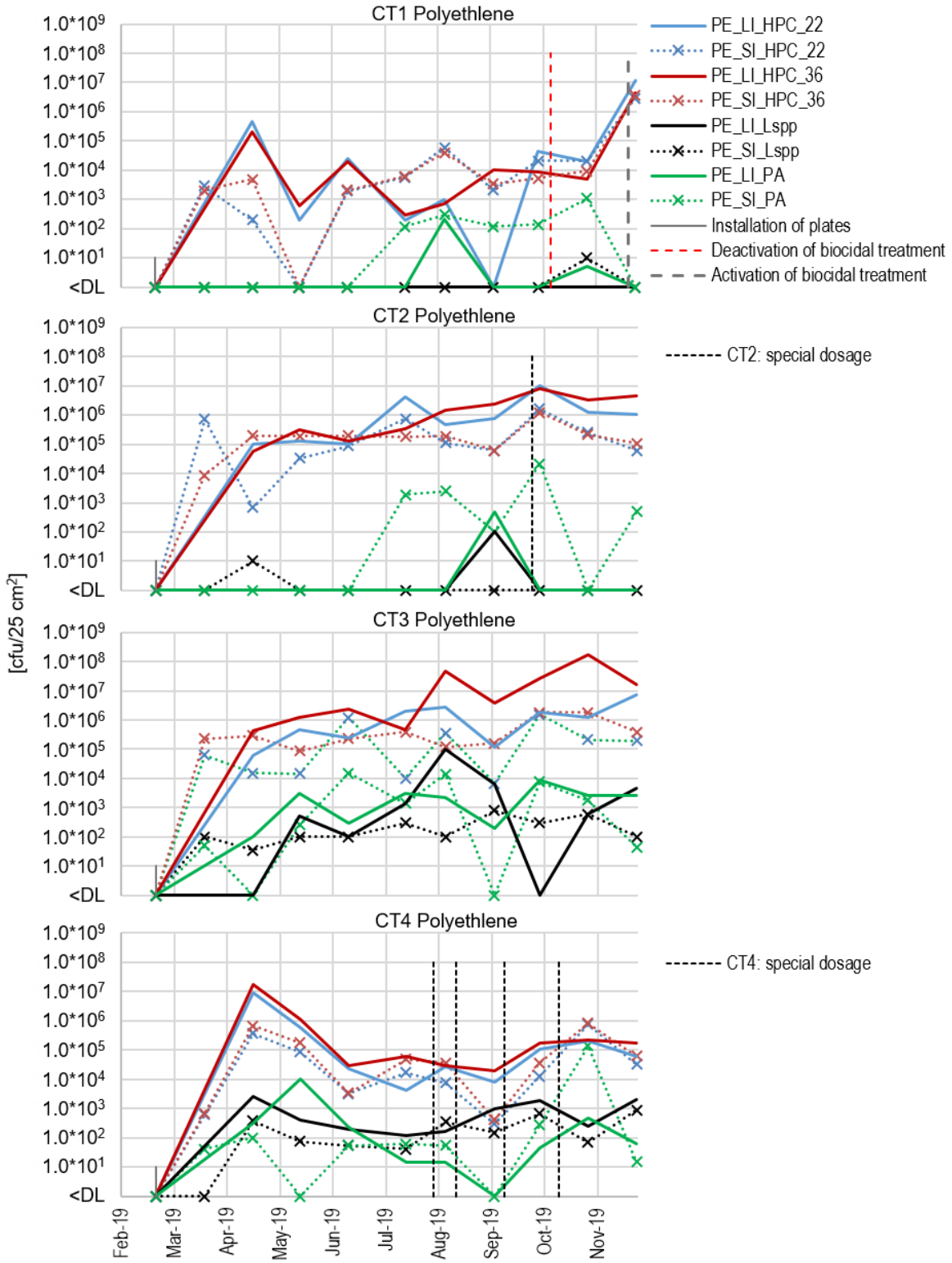


Figure 58: Microbiological composition of the biofilm on the polyethylene plates from the four cooling towers.

PE_LI: long interval polyethylene plate; PE_SI: short interval polyethylene plate.

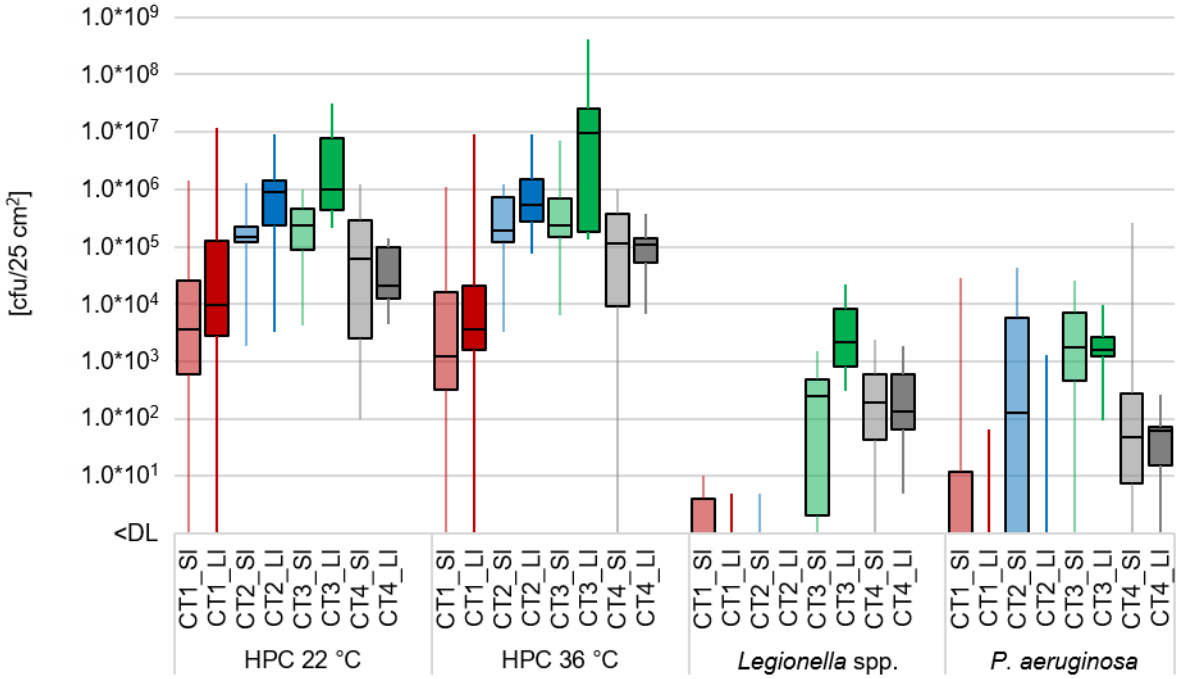


Figure 59: Boxplots of the microbiological parameters grown in biofilms on the stainless steel plates from the four cooling towers.
SI: short interval , LI: long interval.

Table 47: Boxplot values of the microbiological parameters in the biofilm of the stainless steel plates in the four cooling towers.
SI: short interval , LI: long interval, DL: detection limit.

[cfu/25 cm²]	HPC 22 °C		HPC 36 °C		*Legionella* spp.		*P. aeruginosa*	
	SI	LI	SI	LI	SI	LI	SI	LI
Cooling Tower 1								
Maximum	1.4*10⁶	1.2*10⁷	1.1*10⁶	9.3*10⁶	10	5	2.8*10⁴	65
Q3 75 %	2.5*10⁴	1.3*10⁵	1.6*10⁴	2.1*10⁴	4	<DL	12	<DL
Median	3,600	9,500	1,200	3,600	<DL	<DL	<DL	<DL
Q1 25 %	600	2,800	325	1,600	<DL	<DL	<DL	<DL
Minimum	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Cooling Tower 2								
Maximum	1.3*10⁶	9.3*10⁶	1.2*10⁶	9.0*10⁶	5	<DL	4.3*10⁴	1,300
Q3 75 %	2.2*10⁵	1.4*10⁶	7.2*10⁵	1.5*10⁶	<DL	<DL	5.825	<DL
Median	1.5*10⁵	9.1*10⁵	1.9*10⁵	5.3*10⁵	<DL	<DL	126	<DL
Q1 25 %	1.2*10⁵	2.3*10⁵	1.2*10⁵	2.7*10⁵	<DL	<DL	<DL	<DL
Minimum	1.800	3.300	3.300	7.4*10⁴	<DL	<DL	<DL	<DL
Cooling Tower 3								
Maximum	1.0*10⁶	3.1*10⁷	6.9*10⁶	4.2*10⁸	1.500	2.2*10⁴	2.6*10⁴	9,700
Q3 75 %	4.5*10⁵	7.7*10⁶	7.0*10⁵	2.6*10⁷	475	8,200	7,150	2,700
Median	2.4*10⁵	1.0*10⁶	2.4*10⁵	9.4*10⁶	250	2,200	1,760	1,600
Q1 25 %	8.8*10⁴	4.4*10⁵	1.4*10⁵	1.8*10⁵	2	800	450	1,200
Minimum	4,300	2.1*10⁵	6,500	1.3*10⁵	<DL	300	<DL	95
Cooling Tower 4								
Maximum	1.2*10⁶	1.4*10⁵	9.7*10⁵	3.8*10⁵	2,400	1,800	2.6*10⁵	260
Q3 75 %	2.9*10⁵	9.8*10⁴	3.8*10⁵	1.4*10⁵	599	600	270	70
Median	6.2*10⁴	2.1*10⁴	1.2*10⁵	1.1*10⁵	190	130	48	60
Q1 25 %	2,500	1.2*10⁴	8.900	5.2*10⁴	44	65	8	15
Minimum	100	4,400	<DL	6.700	<DL	5	<DL	<DL

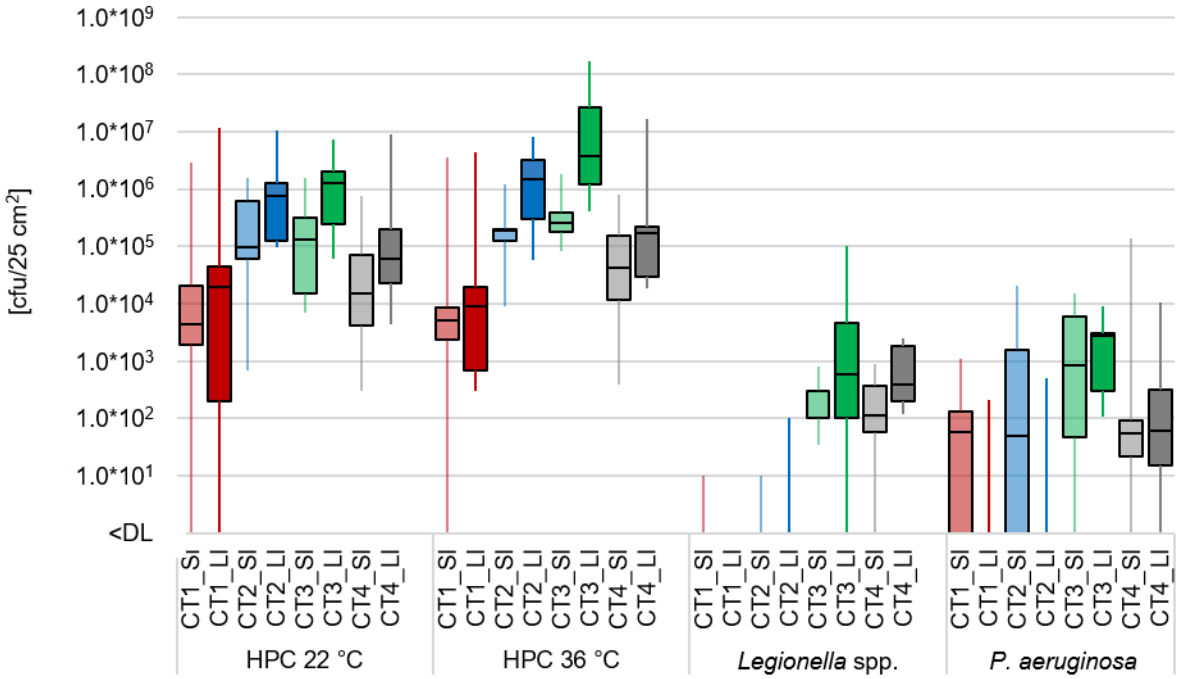


Figure 60: Boxplots of the microbiological parameters grown in biofilms on the polyethylene plates in the four cooling towers.
LI: long interval; SI: short interval.

Table 48: Boxplot values of the microbiological parameters in the biofilm of the polyethylene plates in the four cooling towers.
SI: short interval , LI: long interval, DL: detection limit.

[cfu/25 cm²]	HPC 22 °C		HPC 36 °C		*Legionella* spp.		*P. aeruginosa*	
	SI	LI	SI	LI	SI	LI	SI	LI
Cooling Tower 1								
Maximum	2.9*10⁶	1.2*10⁷	3.6*10⁶	4.5*10⁶	10	<DL	1,100	215
Q3 75 %	2.1*10⁴	4.5*10⁴	8,600	2.0*10⁴	<DL	<DL	134	<DL
Median	4,300	2.0*10⁴	5,050	8,900	<DL	<DL	58	<DL
Q1 25 %	1,950	200	2,400	700	<DL	<DL	<DL	<DL
Minimum	<DL	<DL	<DL	300	<DL	<DL	<DL	<DL
Cooling Tower 2								
Maximum	1.6*10⁶	1.1*10⁷	1.2*10⁶	8.3*10⁶	10	100	1.5*10⁴	500
Q3 75 %	6.1*10⁵	1.3*10⁶	2.0*10⁵	3.2*10⁶	<DL	<DL	5.825	<DL
Median	1.0*10⁵	7.7*10⁵	1.9*10⁵	1.5*10⁵	<DL	<DL	51	<DL
Q1 25 %	6.1*10⁴	1.3*10⁵	1.2*10⁵	3.1*10⁵	<DL	<DL	<DL	<DL
Minimum	690	9.9*10⁴	8,900	6.0*10⁴	<DL	<DL	<DL	<DL
Cooling Tower 3								
Maximum	1.6*10⁶	7.2*10⁶	1.8*10⁶	1.7*10⁸	800	1.0*10⁵	1.5*10⁴	9,000
Q3 75 %	3.2*10⁵	2.0*10⁶	3.9*10⁵	2.7*10⁷	300	4,600	6,075	3,000
Median	1.3*10⁵	1.3*10⁶	2.6*10⁵	3.8*10⁶	100	600	830	2,700
Q1 25 %	1.5*10⁴	2.4*10⁵	1.8*10⁵	1.2*10⁶	100	100	46	300
Minimum	6,900	6.0*10⁴	8.5*10⁴	4.2*10⁵	35	<DL	<DL	105
Cooling Tower 4								
Maximum	7.6*10⁵	9.2*10⁶	8.2*10⁵	1.7*10⁷	875	2,500	1.4*10⁵	1.0*10⁴
Q3 75 %	7.3*10⁴	2.0*10⁵	1.5*10⁵	2.2*10⁵	364	1,800	90	315
Median	1.5*10⁴	6.0*10⁴	4.3*10⁴	1.7*10⁵	113	385	55	60
Q1 25 %	4,250	2.3*10⁴	1.1*10⁴	3.0*10⁴	59	195	21	15
Minimum	300	4.300	400	1.9*10⁴	<DL	120	<DL	<DL

Analysing the data sets of the two plate types of the same investigation interval of each cooling tower with the Wilcoxon signed-rank test, *P. aeruginosa* concentrations did not differ significantly on the polyethylene and stainless steel plates.

Statistical analyses with the Mann-Whitney-U-test of the *P. aeruginosa* data sets of one plate type for both investigation intervals performed individually for each cooling tower provided the result that the *P. aeruginosa* concentrations did not differ significantly in any cooling tower ($p > 0.05$).

Considering the data sets of the same observation interval and plate type, Cooling Towers 1 and 2 did not differ significantly. The data sets of the stainless steel plates of both intervals and the data sets of the long-term polyethylene plates of Cooling Towers 1 and 3 differed significantly. For the data sets of the long-term stainless steel and polyethylene plates of Cooling Towers 1 and 4, 2 and 3 and 3 and 4 a significant difference was obtained with the Mann-Whitney-U-test. The data sets of Cooling Towers 2 and 4 differed significantly only for the long-term polyethylene plates.

3.3.5 Comparative analyses of ISO and IDEXX methods in 40 cooling tower water samples

This chapter includes the results of the comparative analyses for the detection of *Legionella* and *P. aeruginosa* in the 40 cooling water samples using ISO and IDEXX methods.

3.3.5.1 Comparative analysis of Legiolert and the UBA recommended ISO procedures

For the data sets of the 40 cooling water samples tested over the ten-month period, a qualitative method comparison of positive/negative data pairs was carried out as done in section 3.1. The assessment was done by the Mc Nemar's test (see Table 49). Statistical analyses according to ISO 17994 were performed (shown in Table 50 and Figure 61), although the number of positive data pairs was smaller than recommended by this ISO. Additionally the normal distribution of the data was proven (60) and the Wilcoxon signed-rank test (170) was performed to assess if the data sets differed. The distribution of exceeded test and action value of 42nd BImSchV (1) is shown in Table 51 to Table 54.

Table 49 lists the positive/negative data pairs for Legiolert and the ISO *L. pneumophila* and *Legionella* spp counts. The number of positive and negative samples was the same, both for the max. and best Legiolert results. Thus, Legiolert max. and best results are presented in one single table and not separately. As mentioned in 2.5.1.2, the max. Legiolert result represents the higher value of the two test volumes related to 100 mL, the best result represents the value of the two test volumes with the lower measurement uncertainty.

Table 49: McNemar's test of the 40 cooling tower samples for Legiolert max. vs. modified UBA recommended ISO results for *L. pneumophila* and *Legionella* spp. counts.
L. pneumophila results are on left side of the slash, *Legionella* spp. results are on right side of the slash.
L. pneumophila counts: $\chi^2 = 4.23$, $p = 0.040$ / *Legionella* spp. counts: $\chi^2 = 1.75$, $p = 0.185$.

		Modified UBA rec. ISO 11731 proc. *L. pneumophila* / *Legionella spp.* counts -	Modified UBA rec. ISO 11731 proc. *L. pneumophila* / *Legionella spp.* counts +	
Legiolert (max. and best)	-	8 / 8	3 / 3	11 / 11
	+	7 / 4	22 / 25	29 / 29
		15 / 12	25 / 28	40

When comparing the Legiolert results with those of the ISO method for *L. pneumophila* counts, 22 of the 40 samples were quantifiable with both methods (55.0 %). When comparing the Legiolert results with those of the ISO method for *Legionella* spp, 25 of the 40 samples were quantifiable with both methods (62.5 %). Eight samples were negative with both methods for both ISO *L. pneumophila* and *Legionella* spp counts (20.0 %). A negative result with the ISO method, but a positive result with Legiolert was recorded in seven samples (17.5 %) when looking at the ISO *L. pneumophila* results, and in four samples (10 %) when looking at the ISO *Legionella* spp. results. A negative result with Legiolert and a positive result with the ISO method was observed in three samples, both for ISO *L. pneumophila* and for *Legionella* spp. counts.

The McNemar's test indicated that the methods are different ($p = 0.040$) regarding the ISO *L. pneumophila* results. Considering the ISO *Legionella* spp. results the Mc Nemar's test indicated that the methods are not different ($p = 0.185$).

With 32 data pairs each, the data sets for the ISO 17994 analyses were too small to obtain statistically unambiguous statements (see Table 50). Nevertheless, the mean relative difference approach was performed. Figure 61 shows the positions of the "confidence intervals". When looking at the position of the "confidence intervals", it is reasonable to assume that if more samples had been examined Legiolert could have given significantly higher results compared to ISO *L. pneumophila* results. This is assumed, because the "confidence intervals" were shifted into the positive range. When considering Legiolert with ISO *Legionella* spp. results, it seemed that the results are not different since the "confidence intervals" flanked the zero-line equally.

Table 50: Comparative analysis according to ISO 17994 of paired data from Legiolert and *L. pneumophila* and *Legionella* spp. counts from modified UBA recommended ISO procedures.

[a] Half width of the "confidence interval" around the mean relative difference. [b] Value of the relative difference at the lower "confidence limit". [c] Value of the relative difference at the upper "confidence limit".

Data	Number of results	Mean relative difference [%]	Standard deviation [%]	W [a] [%]	X_L [b] [%]	X_U [c] [%]	Outcome
(a) *L. pneumophila* counts from modified UBA recommended ISO 11731:2017 procedures							
Legiolert max. vs ISO	32	74.4	225.3	79.5	-5.2	154.1	Inconclusive
Legiolert best vs. ISO	32	55.5	227.6	80.5	-25.0	136.0	Inconclusive
(b) *Legionella* spp. counts from modified UBA recommended ISO 11731:2017 procedures							
Legiolert max. vs. ISO	32	-9.16	214.0	75.7	-84.8	66.5	Inconclusive
Legiolert best vs. ISO	32	-28.1	225.0	79.5	-107.6	51.5	Inconclusive

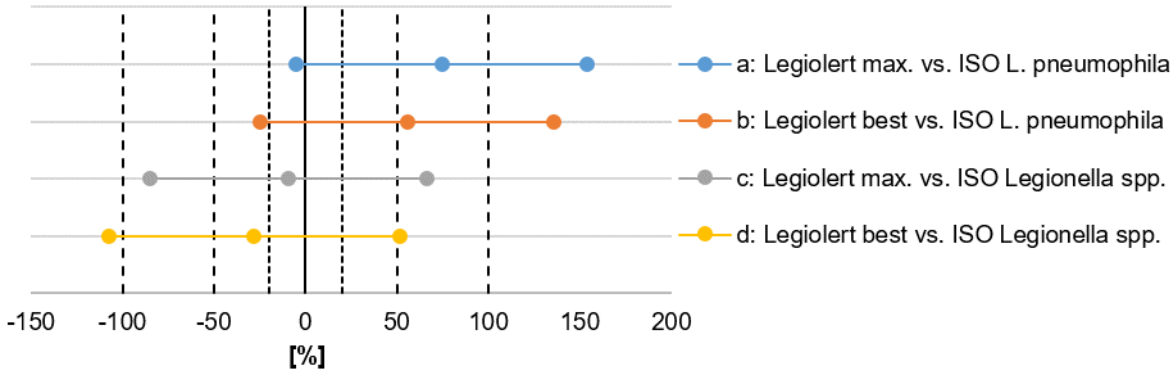


Figure 61: Location of the confidence intervals from comparative analyses according to ISO 17994 of paired data from Legiolert and *L. pneumophila* or *Legionella* spp. results of modified UBA recommended ISO procedures.

For an error probability of 5 % and a two-tailed hypothesis, the Wilcoxon signed-rank test indicated that the ISO *L. pneumophila* and Legiolert max. data sets differed significantly, but the other data sets did not differ significantly.

The paired data comparison of the 40 samples with the test and action values of the 42nd BImSchV is listed in Table 51 to Table 54.

Considering the paired data of Legiolert max. and ISO *L. pneumophila* counts (see Table 51), 30 samples (75.0 %) were in the same value range. Nine samples (22.5 %) exceeded a higher value with Legiolert than the ISO method. One sample exceeded a higher value (2.5 %) with the ISO method. The McNemar's test indicated that the methods are different (p=0.007). Hence, regarding the number of samples exceeding a higher test/action value, it was concluded that Legiolert max. results exceeded significantly more often a higher test/action value than ISO *L. pneumophila* ones.

Comparing the classification of Legiolert best and ISO *L. pneumophila* data pairs (see Table 52) 30 samples (75.0 %) were in the same value range as well. Seven samples (17.5 %) exceeded a higher value with Legiolert than with the ISO method. Three samples exceeded a higher value (7.5 %) with the ISO method. The McNemar's test indicated that the methods are not different (p = 0.155) regarding the number of samples exceeding a higher test/action value with one method.

Table 51: Classification of Legiolert max. and ISO *L. pneumophila* results according to 42nd BImSchV.

Mc Nemar's test: $\chi 2 = 7.225$, p-value = 0.007.

		ISO 11731 *L. pneumophila*				
		≤ Test Value 1	> Test Value 1	> Test Value 2	> Action Value	
Legiolert max.	≤ Test Value 1	**17**	1	0	0	18
	> Test Value 1	6	**7**	0	0	13
	> Test Value 2	0	1	**4**	**0**	5
	> Action Value	0	0	2	**2**	4
		23	9	6	2	40

Table 52: Classification of Legiolert best. and ISO *L. pneumophila* results according to 42nd BImSchV.

Mc Nemar's test: $\chi 2 = 2.025$, p-value = 0.155.

		ISO 11731 *L. pneumophila*				
		≤ Test Value 1	> Test Value 1	> Test Value 2	> Action Value	
Legiolert best.	≤ Test Value 1	**18**	2	0	0	20
	> Test Value 1	5	**7**	1	0	13
	> Test Value 2	0	0	**3**	0	3
	> Action Value	0	0	2	**2**	4
		23	9	6	2	40

Table 53: Classification of Legiolert max. and ISO *Legionella* spp. results according to 42nd BImSchV.

Mc Nemar's test: $\chi 2 = 1.36$, p-value = 0.243.

		ISO 11731 *Legionella* spp.				
		≤ Test Value 1	> Test Value 1	> Test Value 2	> Action Value	
Legiolert max.	≤ Test Value 1	**17**	1	0	0	18
	> Test Value 1	4	**7**	2	0	13
	> Test Value 2	0	1	**4**	0	5
	> Action Value	0	0	1	**3**	4
		21	9	7	0	40

Table 54: Classification of Legiolert best and ISO *Legionella* spp. results according to 42nd BImSchV.

Mc Nemar's test: $\chi^2 = 0.027$, p-value = 0.868.

		ISO 11731 *Legionella* spp.				
		≤ Test Value 1	> Test Value 1	> Test Value 2	> Action Value	
Legiolert best	≤ Test Value 1	**18**	2	0	0	20
	> Test Value 1	3	**7**	3	0	13
	> Test Value 2	0	0	**3**	0	3
	> Action Value	0	0	1	**3**	4
		21	9	7	3	40

The classification of Legiolert max. and ISO *Legionella* spp. data pairs (see Table 53) showed that 31 samples (77.5 %) were in the same value range. Six samples (15.0 %) exceeded a higher value with Legiolert than with the ISO method. Three samples exceeded a higher value (7.5 %) with the ISO method. The McNemar's test indicated that the methods are not different (p = 0.243) regarding the number of samples exceeding a higher test/action value with one method.

The classification of Legiolert best and ISO *Legionella* spp. data pairs (see Table 54) showed that 31 samples (77.5 %) were in the same value range as well. Four samples (10.0 %) exceeded a higher value with Legiolert than with the ISO method. Five samples exceeded a higher value (12.5 %) with the ISO method. The McNemar's test indicated that the methods are not different (p=0.868) regarding the number of samples exceeding a higher test/action value with one method.

The influence of the measurement uncertainty on the final result is shown in Figure 62. With the ISO method, more results were obtained influenced by a low measurement uncertainty.

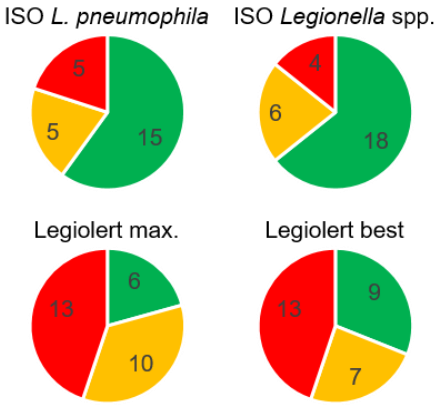


Figure 62: Measurement uncertainty of ISO and Legiolert counts of the water samples from the biofilm experiments.
Green: cfu or positive wells ≥ 10, low measurement uncertainty (MU); orange: 10 > cfu or positive wells ≥ 3, high MU; red: cfu or positive wells < 3, very high MU

As shown in Table 49, *Legionella* spp. was detected in 28 samples. The distribution of modified UBA recommended ISO procedures providing the final result are illustrated in Figure 63. Ten of the results were obtained from the membrane filtration with acid treatment procedure, twelve from the direct plating after heat treatment procedure and six from the direct-plating after acid treatment procedure.

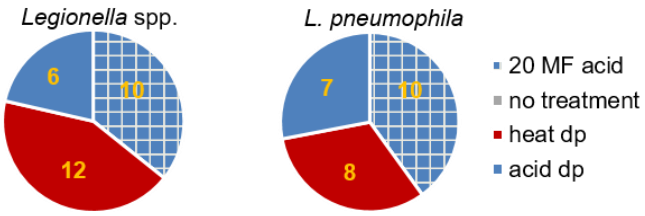


Figure 63: Distribution of the modified UBA rec. ISO procedures for the final result calculation.
MF: membrane filtration; dp: direct plating.

L. pneumophila was detected in 25 samples. Ten of the results were obtained from the membrane filtration with acid treatment procedure, eight from the direct plating after heat treatment procedure and seven from the direct plating after acid treatment procedure. No final result originated from the direct plating without pretreatment procedure.

3.3.5.2 Comparative analysis of Pseudalert and ISO 16266 for the detection of *P. aeruginosa*

In this section, the results of the comparative analysis of Pseudalert and the ISO method for the 40 cooling tower water samples are shown. The number of positive/negative paired data is described, the results of the comparative analysis according to ISO 17994 (79) are presented, the outcome of the Wilcoxon signed-rank test is shown and a comparative description is given of the measurement uncertainty that influenced the results of both methods. Due to the frequent results above the upper quantification level of *P. aeruginosa* in the water samples of Cooling Tower 4 from the 10 mL membrane filtration, 1 mL (2 * 0.5 mL) was directly plated on Cetrimide agar, although this procedure does not correspond to the requirements of the ISO method.

Of the 40 samples tested with both methods, 23 samples were positive and twelve samples were negative with both methods as shown in Table 55.

Table 55: Mc Nemar's Test of the 40 cooling tower samples for Pseudalert vs. ISO results
One sample result exceeded the UQL with the ISO method (quantifiable with Pseudalert) $\chi^2 = 1.56$, $p = 0.211$.

		ISO 16266 -	ISO 16266 +	
Pseudalert	-	12	1	13
	+	3	23	26
		15	24	39

One sample was quantifiable with the ISO method but negative with Pseudalert. Three samples showed quantifiable results with Pseudalert but negative results with the ISO method. One sample is not listed in Table 55. It exceeded the upper quantification level of the ISO method while it was quantifiable with Pseudalert. In total 27 samples were positive with Pseudalert. With a Chi-square value of 1.56 and a p-value of 0.211 the McNemar's test indicated that the methods are not different.

With 27 data pairs the data set for the ISO 17994 analyses were too small to obtain statistically unambiguous statements (see Table 56). Nevertheless, the analysis was performed and gave an inconclusive outcome. The mean relative difference amounted to 2.7 %. The upper limit of the "confidence interval" was 62.6 %, the lower limit -58.1 %. Figure 64 shows the position of the "confidence interval" indicating that the methods are equal since the zero line was flanked equally. For an error probability of 5 % and a two-sided consideration, the Wilcoxon signed-rank test (170) showed that the data sets are not significantly different.

Table 56: Comparative analysis of paired data from Pseudalert and the ISO method.
[a] Half width of the "confidence interval" around the mean relative difference. [b] Value of the relative difference at the lower "confidence limit". [c] Value of the relative difference at the upper "confidence limit".

Number of results	Mean relative difference [%]	Standard deviation [%]	W [a] [%]	X_L [b] [%]	X_U [c] [%]	Outcome
27	2.7	153.0	58.9	-56.1	62.6	Inconclusive

According to equation 5.4.3 shown in ISO 17994 additional 340 samples would have to be tested to gain an statistically clear result (79).

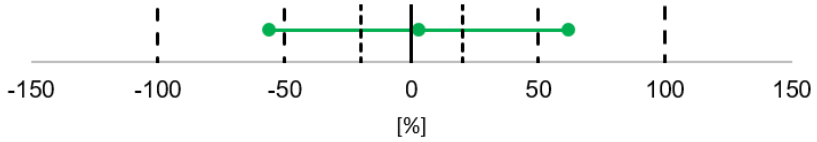


Figure 64: Location of the confidence interval from relative differences of the comparative analysis according to ISO 17994 of paired data from Pseudalert and the ISO method.

Of the 27 samples tested positive for *P. aeruginosa* by Pseudalert, 19 results were influenced by a low measurement uncertainty, four by a high one and also four by a very high measurement uncertainty. Of the 24 samples tested positive for *P. aeruginosa* by the ISO method, 16 results were influenced by a low measurement uncertainty, three results were influenced by a high measurement uncertainty and five by a very high measurement uncertainty.

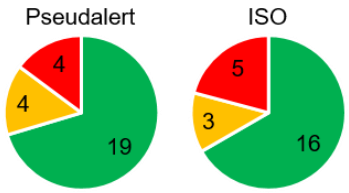


Figure 65: Measurement uncertainty of Pseudalert and ISO counts.
Green: cfu or positive wells ≥ 10, low measurement uncertainty (MU); orange: 10 > cfu or positive wells ≥ 3, high MU; red: cfu or positive wells < 3, very high MU.

3.4 Biofilm formation in a cooling tower under deactivated biocide treatment conditions

During the observation period, the unique opportunity arose to observe the biofilm development in Cooling Tower 1 under deactivated biocide treatment. From October 7th to November 21st 2019 the biocide treatment was switched off. During this period, water samples were analysed weekly for the presence of HPC, *Legionella* spp. and *P. aeruginosa*. Two plate holding units were placed in the cooling tower basin on October 7th, where the other plate holders of the ten-month experiment were also located. Also in this study the long-term and short-term monitoring was performed. The long-term plates remained in the cooling tower basin since October 7th. The plates were removed in a biweekly rhythm parallel. The first plate removal after installation was already carried out after one week. After reactivation of the biocide treatment, the plates were removed twice after three weeks. Nine weeks passed from the second last to the last removal. At each removal, a clean and disinfected pair of plates was placed in the plate holding unit for the short-term observation. The biocide dosing is carried out automatically depending on the redox potential. The curve of the redox potential is shown in Figure 66.

The water samples and swabs were analysed for HPC, *Legionella* spp. and *P. aeruginosa*. In contrast to section 3.3, the internal laboratory method approved by the UBA was used to detect *Legionella* spp. in water samples (procedure described in 2.5.1.1.3 on page 43). In addition to the ISO agar plating cultivation methods, the water samples were tested for *L. pneumophila* and *P. aeruginosa* with the IDEXX most probable number cultivation methods Legiolert and Pseudalert. In Figure 67, the curves of the different parameters tested in the water samples are shown. In Table 57, the corresponding concentrations are listed. The results of the plate smears are shown in Figure 68 and Table 58. In Figure 69, the results of the different parameters in the water samples and on the plates are shown in four charts.

Figure 67 shows HPC and *Legionella* spp. results from July 2019 on to demonstrate the course of concentrations prior to biocide deactivation. The cooling tower was routinely monitored for HPC and *Legionella* spp. but not for *P. aeruginosa* by the Laboratory for Technical Hygiene. Within the biofilm experiments, tests for *P. aeruginosa* (ISO method, Pseudalert) and Legiolert were carried out in July and August. As the date of examination of routine monitoring and study samples was not the same, the results were not included. In September, plate sampling and routine monitoring were performed on the same day, so that the results of the "non-routine parameters" are shown. In addition to the curves of the microbiological parameters, the figure shows the times of deactivation of the biocide treatment (red dashed vertical line) and reactivation (grey dashed vertical line) as well as the reference values of the HPC according to 42nd BImSchV for 22 °C (pale blue horizontal line) and 36 °C (pale red horizontal line).

3.4.1 Redox potential curve before, during and after deactivated biocide treatment

Figure 66 illustrates the course of the redox potential. The upper chart shows the period from October 1st to December 31st. The orange line indicates the deactivation of the biocide treatment, the green line the reactivation. The time around deactivation is shown in the lower left-hand chart, around reactivation in the lower right-hand chart. Usually, biocide dosing was initiated when the redox potential fell below the threshold value of 400 mV. The presence of chlorine dioxide caused the redox potential to rise. As soon as a redox potential of 401 mV was registered at the electrode, the dosing stopped. Since the dosing pump is located at a different point in the cooling tower than the electrode, there is a time delay and a higher redox potential was generated in the system when 401 mV is measured at the electrode. Before deactivating the biocide treatment, the lower left chart shows that two dosages per day were carried out. After the biocide treatment was stopped, the redox potential oscillated between 430 and 450 mV until October 9th. The decrease of the curve is due to the exchange of the electrode. On October 13th the biocide treatment was accidentally reactivated and two dosages were carried out indicated by two peaks in the upper chart. Afterwards, it is visible that the redox potential has levelled off again below 400 mV. Differences in the horizontal redox potential lines occurred after the replacement of electrodes. When reactivating the biocide dosage, three peracetic acid shock dosages were initially carried out, which are not visible in the figure. Afterwards chlorine dioxide was dosed again. Due to a faulty electrode, no chlorine dioxide doses were carried out between 23rd and 25th November. From 26th the dosing worked trouble-free.

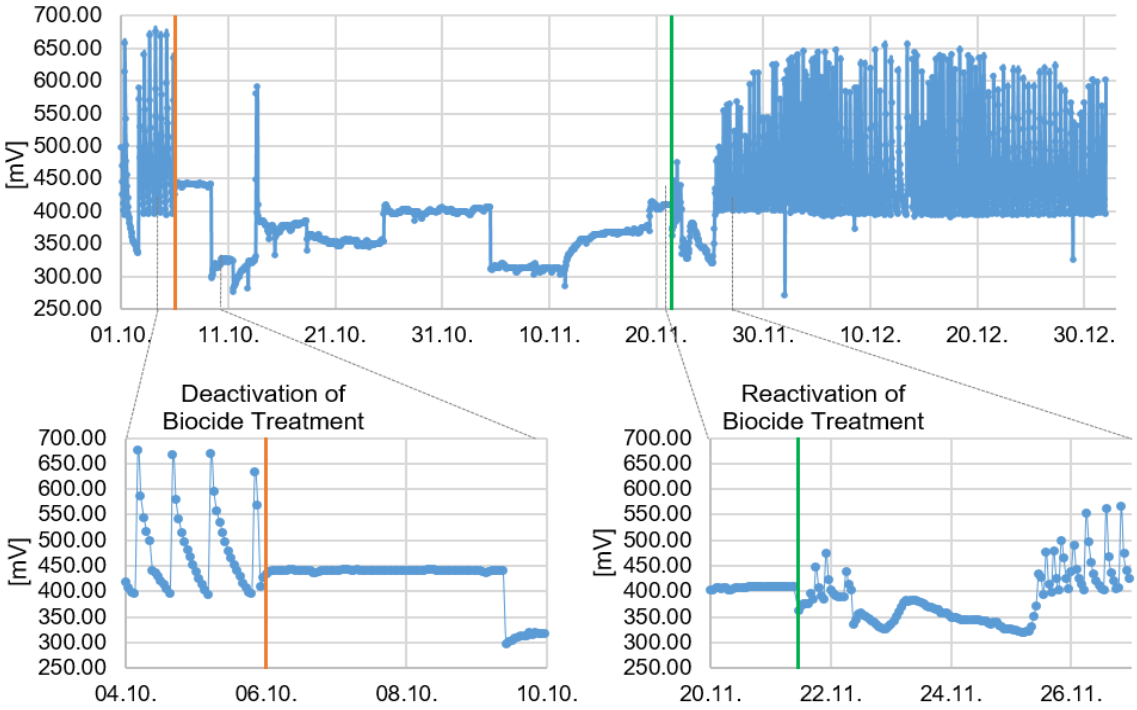


Figure 66: Redox potential before and during deactivation and after reactivation of the biocide treatment.
Orange: deactivation of biocide treatment; green: reactivation of biocide treatment.

3.4.2 Microbial concentrations in water samples

The HPC fluctuated before the biocide deactivation. In the July monitoring, counts lower than the reference values were determined. The reference value for HPC at 22 °C amounted to 452 cfu/mL and for HPC at 36 °C to 213 cfu/mL. In August, counts were obtained that exceeded the reference values by a factor of almost 10. In the following month, a decrease of HPC below the detection limit was observed. At the time of biocide deactivation, values in the range of the July monitoring were detected. During the time without biocide treatment, HPC fluctuated within a log level. HPC at 22 °C seemed to stagnate almost during this period, whereas HPC at 36 °C gradually increased. The reference values were only exceeded once. This was not a case of exceeding the 100-fold reference value causing actions according to the 42nd BImSchV. After reactivation of the biocide treatment, HPC increased rapidly by a factor of almost 1,000 (complaint according to 42nd BImSchV). At the beginning of December, HPC were recorded at concentrations similar to those at the time of deactivated biocide treatment. Within the next two weeks, a slight increase was recorded, followed by a steady decrease until February. Until the last recording in March, HPC stagnated at 36 °C while HPC at 22 °C rose to the level during deactivated biocide treatment.

Legionellae were absent or detected in very low concentrations before biocide treatment deactivation. The highest concentrations were determined during biocide deactivation by both methods. Using the ISO method, first an increase and then stagnation in the lower hundred range was observed until reactivation. With Legiolert, *L. pneumophila* was not detected one week after deactivation, and the subsequent investigation was omitted. From the end of October until the last monitoring before reactivation, by Legiolert concentrations between 100 and 1,000 mpn/100 mL were determined. After reactivation of the biocide treatment, *Legionella* spp. or *L. pneumophila* were not detected in the next two tests by both methods. Subsequently, low concentrations below 100 cfu or mpn/100 mL or no legionellae were detected. All detected legionellae colonies belonged to *L. pneumophila* from the serogroup range 2 - 14. At this point it should be mentioned again that the tests for *Legionella* spp. of this study part were carried out with the accredited method of the Laboratory for Technical Hygiene and not with the modified UBA method of the “Biofilm study part” (section 3.3) which detected a *L. species* strain on October 28th. The figure gives the impression that *L. pneumophila* was detected in the February investigation with Legiolert, but not with the ISO method. In fact, in February the examination with Legiolert was not carried out, as shown in Table 57.

P. aeruginosa was the least common and the lowest detected organism. Before deactivation, the bacterium was detected at low concentrations by the ISO method, while Pseudalert was negative. During the deactivated biocide treatment, no detection or a detection corresponding to the lower limit of quantification occurred. With both methods, the highest *P. aeruginosa*

concentrations were recorded at sampling after reactivation. Subsequently, the concentrations decreased again and *P. aeruginosa* was not detected anymore with the ISO method from January onwards. By Pseudalert sporadically detection occurred, but Pseudalert was not performed in February.

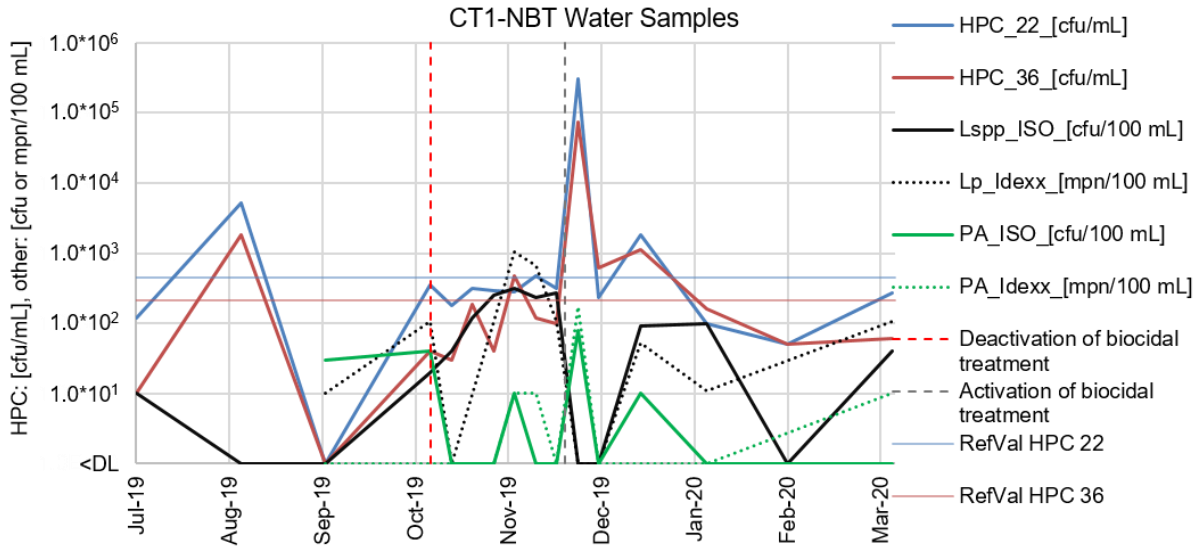


Figure 67: CT1 - Deactivated biocide treatment: microbial composition of the water samples.

Table 57: CT1 - Deactivated biocide treatment: microbial concentrations in water samples.
Concentrations obtained during the deactivated biocide treatment are shown in bold.

Datum	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	*Legionella* spp. ISO [cfu/100 mL]	*L. pneumophila* IDEXX [mpn/100 mL]	*P. aeruginosa* ISO [cfu/100 mL]	*P. aeruginosa* IDEXX [mpn/100 mL]
01.07.2019	120	10	10	-	-	-
05.08.2019	5,100	1,800	<DL	-	-	-
02.09.2019	<DL	<DL	<DL	10	30	<DL
07.10.2019	**350**	**40**	**20**	**108**	**40**	**<DL**
14.10.2019	**180**	**30**	**41**	**<DL**	**<DL**	**<DL**
21.10.2019	**320**	**190**	**120**	**-**	**<DL**	**-**
28.10.2019	**290**	**40**	**250**	**108**	**<DL**	**<DL**
04.11.2019	**280**	**470**	**310**	**1,038**	**10**	**10**
11.11.2019	**480**	**120**	**230**	**656**	**<DL**	**10**
18.11.2019	**320**	**100**	**270**	**108**	**<DL**	**<DL**
25.11.2019	$3.1*10^5$	$7.3*10^4$	<DL	<DL	80	171
02.12.2019	230	620	<DL	<DL	<DL	<DL
16.12.2019	1,800	1,110	91	52	10	<DL
07.01.2020	100	160	100	11	<DL	<DL
03.02.2020	50	50	<DL	-	-	-
09.03.2020	270	60	40	108	<DL	10

3.4.3 Microbial composition of the biofilms grown on stainless steel and polyethylene plates

In this section, the composition of the biofilms grown on the plates is presented. In Figure 68, the curves of the different parameters for the stainless steel and polyethylene plates are shown. At first sight, the charts look very similar. During the deactivated biocide treatment, increasing HPC were recorded on both plate materials for the short interval. Within the first week, HPC were determined between 8,800 and $3.5 * 10^4$ cfu/25 cm^2. The highest HPC were observed with values from $3.5 * 10^5$ to $5.7 * 10^5$ cfu/25 cm^2 on the plates of the short observation period in the last investigation before reactivation of the biocide treatment. Within this period, the heterotrophic bacteria of the long observation period grew to concentrations in the range of 10^4 to 10^5 cfu/25 cm^2 and tended to stagnate. After reactivation of the biocide and peracetic acid treatment, the HPC on the plates were strongly reduced. The HPC of both incubation temperatures of the polyethylene plates of both observation intervals and the HPC at 36 °C of the short- and long-term stainless steel plates were reduced to below the detection limit. The HPC at 22 °C of the short-term and long-term stainless steel plates were reduced by the factor of 1,000 compared to the previous removal of plates. At the next plate removal in mid-December, almost identical HPC values were recorded for the stainless steel plates at the level of the long-term stainless steel plates before biocide reactivation, regardless of incubation temperature and observation interval. On the polyethylene plates, HPC at 36 °C of both observation intervals and HPC at 22 °C of the short-term stainless steel plate showed rapidly increasing values in the lower 10^4 cfu/25 cm^2 range. The HPC at 22 °C of the long-term polyethylene plate even exceeded 10^5 cfu/25 cm^2. In the January investigation, increases within one logarithmic level were recorded, except for decreased HPC at 22 °C on the long-term plates. At the last plates removal in early March, decreased values were recorded for both plate types, study intervals and incubation temperatures. On the short interval polyethylene plate, HPC at 36 °C were not detected at all. In total, both plate types were found to have higher HPC at 22 °C than at 36 °C, and the stainless steel plates were found to have higher HPC values more often than the polyethylene plates.

Legionellae were only sporadically detected. The microorganisms were not detected on the short-term stainless steel plates. Once *L. pneumophila* was detected on a long-term stainless steel plate in a concentration of 100 cfu/25 cm^2. On the polyethylene plates of both intervals, *L. pneumophila* was found twice each at the lower limit of quantification. All detected legionellae colonies belonged to *L. pneumophila* from serogroups 2 - 14.

The curves of *P. aeruginosa* were almost congruent for both plate types. Nearly the same concentrations were found on plates of the same observation period. During deactivated biocide treatment, *P. aeruginosa* cfu were detected only on the short-term plates in the lower

hundreds per 25 cm^2. After biocide reactivation, the bacterium was not detected until January. The concentrations were two log_{10} levels higher on both plate types of the short interval than on the long-term plates.

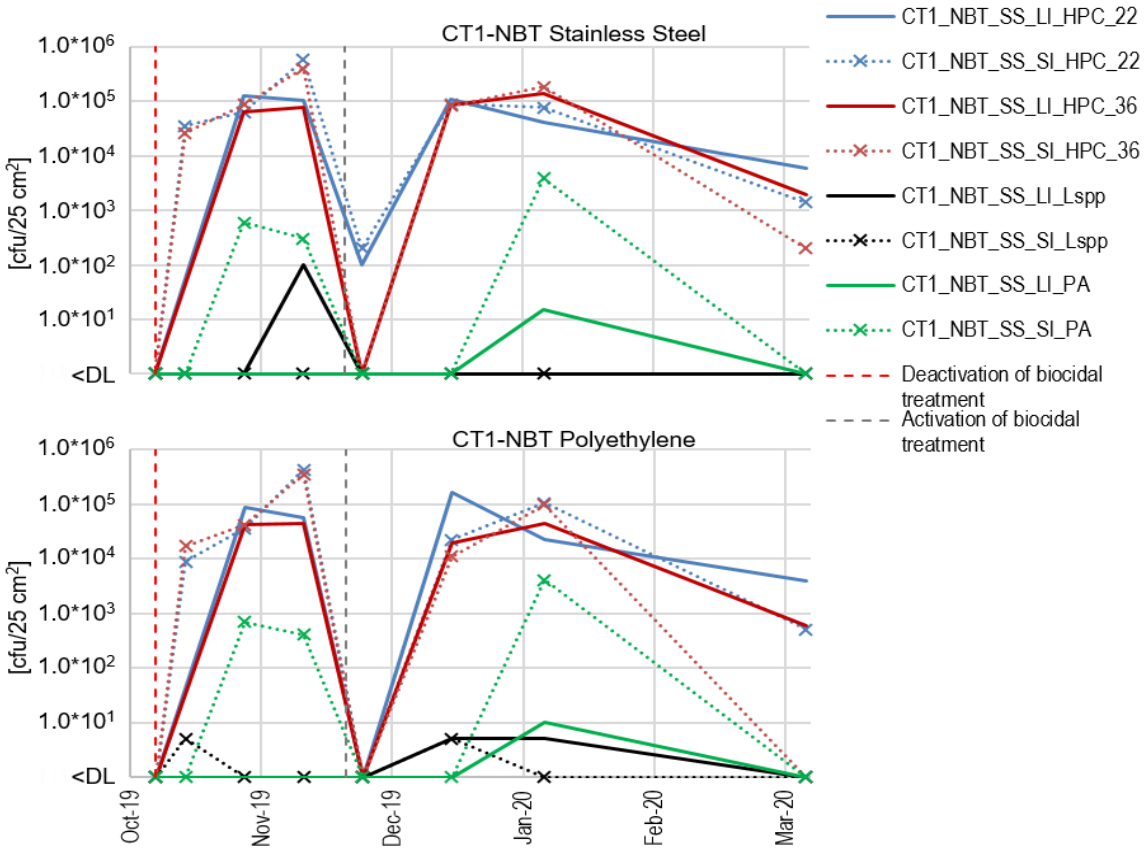


Figure 68: CT1 - Deactivated biocide treatment: microbial growth on plates.
SS_LI: long interval stainless steel plate; SS_SI: short interval stainless steel plate.

Table 58: CT1- Deactivated biocide treatment: microbial concentrations in the biofilms.
DL: detection limit.

a) Stainless steel plates

[cfu/25 cm^2]	Short interval observation				Long interval observation			
	HPC 22 °C	HPC 36 °C	*Legionella* spp.	*P. aerugi-nosa*	HPC 22 °C	HPC 36 °C	*Legionella* spp.	*P. aerugi-nosa*
14.10.2019	$3.5*10^4$	$2.6*10^4$	<DL	<DL	-	-	-	-
28.10.2019	$6.2*10^4$	$8.5*10^4$	<DL	600	$1.2*10^5$	$6.4*10^4$	<DL	<DL
11.11.2019	$5.7*10^5$	$3.9*10^5$	<DL	300	$1.0*10^5$	$7.8*10^4$	100	<DL
25.11.2019	200	<DL	<DL	<DL	100	<DL	<DL	<DL
16.12.2019	$8.8*10^4$	$8.2*10^4$	<DL	<DL	$1.1*10^5$	$8.4*10^4$	<DL	<DL
07.01.2020	$7.6*10^4$	$1.8*10^5$	<DL	3,800	$4.0*10^4$	$1.4*10^5$	<DL	15
09.03.2020	1,400	200	<DL	<DL	5,900	1,900	<DL	<DL
b) Polyethylene plates								
14.10.2019	8,800	$1.7*10^4$	5	<DL	-	-	-	-
28.10.2019	$3.6*10^4$	$4.1*10^4$	<DL	685	$8.5*10^4$	$4.1*10^4$	<DL	<DL
11.11.2019	$4.2*10^5$	$3.5*10^5$	<DL	400	$5.6*10^4$	$4.4*10^4$	<DL	<DL
25.11.2019	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
16.12.2019	$2.2*10^4$	$1.1*10^4$	5	<DL	$1.6*10^5$	$1.9*10^4$	5	<DL
07.01.2020	$1.0*10^5$	$9.7*10^4$	<DL	4,000	$2.2*10^4$	$4.3*10^4$	5	10
09.03.2020	500	<DL	<DL	<DL	3,900	600	<DL	<DL

3.4.4 Microbial distribution in water samples and in biofilms grown on plates

The charts of Figure 69 show the individual parameters determined in the water samples and on the plates. At this point, the individual curves will not be explained here, as this has already been done in sections 3.4.2 and 3.4.3. The purpose of this figure is to show how the individual parameters in the water samples and in the biofilm on the plates have developed during the deactivation of the biocide treatment and after reactivation.

For the charts of HPC at 22 and 36 °C, the curves during and after reactivation are of particular importance. While moderate concentrations without a significant increase in concentration were recorded in the water samples during deactivated biocide treatment, the plates showed impressive concentrations. When comparing these concentrations with those from March to September of the "Biofilm study" in section 3.3.2.1, it is evident that above-average concentrations were determined on both types of plate and for both intervals. After reactivation of the biocide dosage, i.e. after the peracetic acid shock dosages, a drastic reduction of HPC was recorded on all plates, while HPC in the water samples increased by a multiple. In the following investigations of the biofilm suspensions in mid-December, when the chlorine dioxide dosage was again intact, a significant increase in HPC was again recorded on the plates, accompanied by a reduction in the water sample. Until January, the HPC in the biofilms formed on the plates stagnated and in the last investigation in March, reductions of at least one log level were recorded on all plates. The concentrations in the water sample were about the same as during biocide deactivation. The chlorine dioxide was consequently able to counteract biofilm growth from January onwards and to kill microorganisms in the water, or to keep the concentration at the same level.

In the *Legionella* chart, an increase on the plates during the biocide deactivation and in the second sampling after reactivation was also partly observed. As for HPC, the concentration in the water samples increased during the deactivated biocide treatment. However, the growth rates on the plates during biocide deactivation are much lower compared to HPC. Apparently, no ideal conditions were created for *Legionella* or their hosts to colonise in large quantities in Cooling Tower 1 during biocide deactivation. It is not clear whether *Legionella* colonized other areas in the cooling tower in higher quantities, because during reactivation of the biocide treatment, legionellae were apparently immediately killed, as there peak was no in the water sample after reactivation as for HPC.

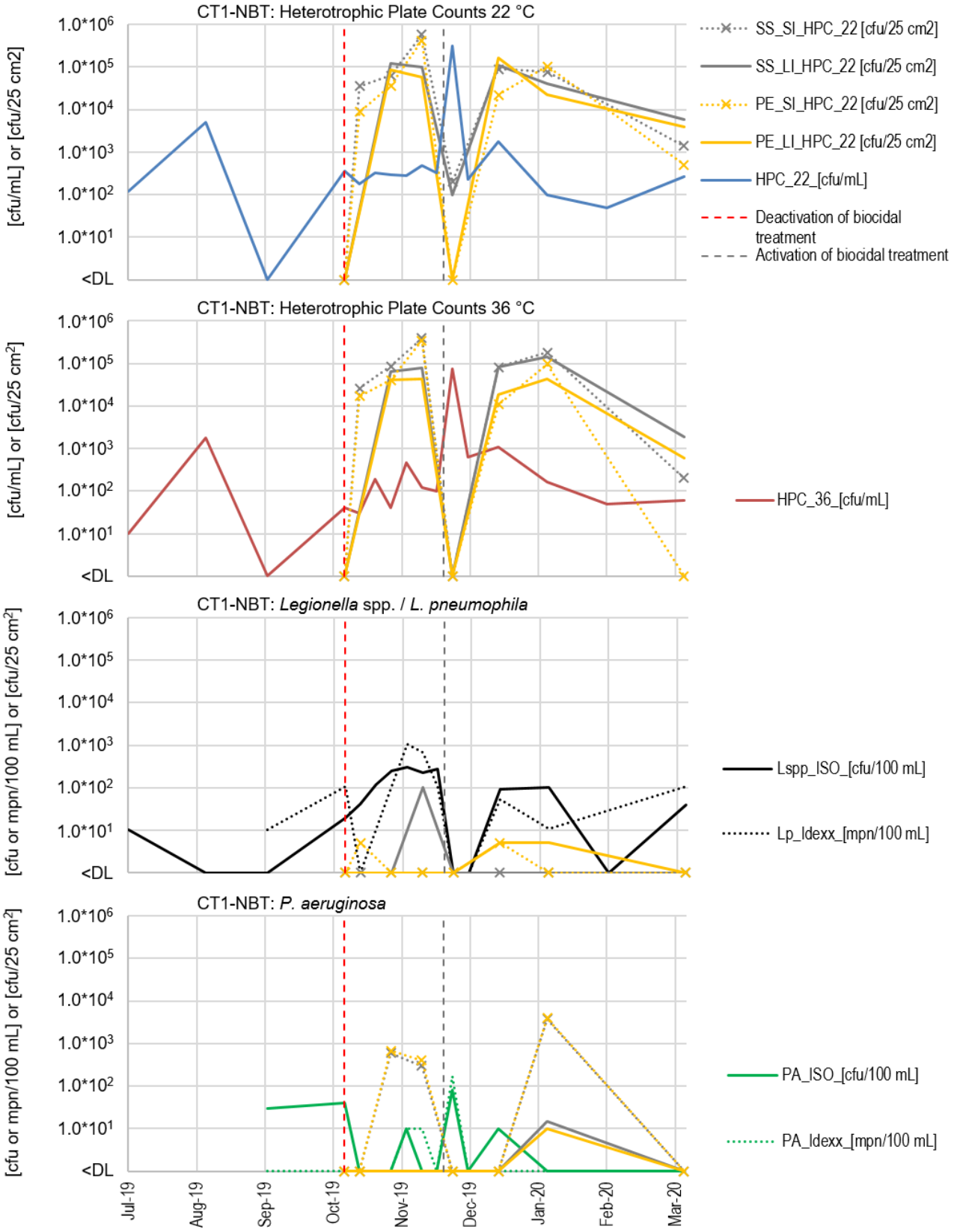


Figure 69: CT1- Deactivated biocide treatment: microbial growth on the stainless steel and polyethylene plates and in the water samples.

SS: stainless steel; PE: polyethylene; xx-LI: long interval plate; xx_SI: short interval.

The ratio of free-living planktonic and sessile biofilm-associated *P. aeruginosa* is similar to the HPC charts. As expected, the bacterium known as first colonizer of surfaces and biofilm founder (162) was detected especially on the plates of the short observation period. The concentrations of planktonic and sessile *P. aeruginosa* were already low in Cooling Tower 1 during the "biofilm experiments" (see section 3.3.2.1), and it is also low during biocide deactivation. After reactivation of biocide treatment and the peracetic dosages, the release of sessile *P. aeruginosa* into the "pelagial" is recorded. Not in mid-December, as with the other parameters, but only in January a stately colonization of *P. aeruginosa* was observed, especially on the plates of the short interval.

During sampling and smear tests, the algae coverage on the cooling tower walls and internals was noted. During the installation of the plate holders and the first sampling after one week, few thin algae films were recorded. On October 21st and 28th, a slight increase in algae was observed. Until the end of November, a strong increase in algae growth was recorded in each sampling. At sampling on November25th, a thick layer of algae covered the walls and internals. On December 2nd, grey dying algae were already noted. On December16th, a large part of the algae had died. The dead algae detached from their carpet. The entry of the dead algae into the cooling water probably bound some oxygen radicals of the chlorine dioxide and served as food for the microorganisms. On January 7th, it was noted that about 75 % of the algae layer had disappeared. In February, the situation was the same as before the biocide deactivation, while in March a slight increase in algae presumably due to warmer temperatures was noted again.

4 Discussion

Due to the risk of the dissemination of legionellae from contaminated cooling towers, it is of enormous importance for the public health that cooling towers are well maintained according to the generally accepted technical regulations. In this context, the understanding of biofilm formation in cooling towers in general and the role of legionellae in particular is of great relevance.

Based on this background, new detection methods for *L. pneumophila* and *P. aeruginosa* were tested for their suitability in order to apply reliable methods for the detection in cooling water samples in addition to the standardized agar cultivation methods. A prediction tool and risk assessment for heterotrophic plate counts and *Legionella* was created using retrospective data from the four investigated cooling towers. Within a long-term study, the biofilm formation was observed in four different, well maintained cooling towers.

4.1 Applicability of methods for the detection of microorganisms in cooling tower water samples

In this section, the suitability of the different microbial methods is evaluated, which have to be performed in cooling water samples according to 42nd BImSchV (1) and VDI Code of Practice 2047-2 (159). The possible alternative methods of IDEXX are evaluated with regard to their applicability. It was examined whether the performance of one single incubation temperature is sufficient in the detection of HPC.

4.1.1 Applicability of Legiolert™/Quanti-Tray® for the detection of *Legionella pneumophila* in cooling tower water

Legiolert was developed for the detection of *L. pneumophila* in water samples. The majority of *Legionella* infections in Europe and the USA are caused by *L. pneumophil*a (41, 163). Thus, a reliable detection of *L. pneumophila* is of great interest for public health. In contrast to Legiolert, the ISO method is also able to detect other *Legionella* species.

In this study, comparative analyses of Legiolert and the (partly modified) UBA recommended ISO procedures in industrial waters were performed. In section 3.1 on page 54 onwards, the results of the comparative analysis according to ISO 17994 of 99 water samples from cooling towers, wet scrubbers and other industrial sites tested by Legiolert and the UBA recommended

ISO procedures are shown. In section 3.3.5.1 on page 152 onwards, the results of the comparative analysis of Legiolert and the modified UBA recommended ISO procedures of the 40 cooling water samples during the biofilm experiments are described.

As shown in section 3.1 Legiolert gave significantly higher results than the *L. pneumophila* counts of ISO method (see Table 13 on page 57).

It is questionable whether a comparison of Legiolert with the *Legionella* spp. counts of the ISO method is really meaningful, since different parameters are considered. On the other hand, it is questionable whether it is even useful to detect other species than *L. pneumophila*, because so far no other *Legionella species* than *L. pneumophila* were detected at legionellosis outbreaks associated with cooling towers and described in the literature (2, 12, 24, 31, 37, 38, 41, 47, 49, 50, 54, 58, 59, 66, 68, 83, 85, 87, 101, 112, 113, 120, 124, 133–135, 138, 164, 167, 169). In addition, the use of GVPC in particular promotes the growth of *L. pneumophila*. Nevertheless, a comparison of Legiolert with the ISO *Legionella* spp. counts was made in this study. The outcome of the ISO 17994 analyses was „inconclusive“ (see Table 14 a on page 59). Consequently, it was statistically verified whether the methods are equivalent or not. The „inconclusive“ outcome is probably based on the consideration of unequal parameters and on the fact that results of different volumes have been extrapolated to mpn or cfu per 100 millilitre. Furthermore, the ISO method has a higher dispersion of the final result due to the higher choice of plates, leading to a bias in the statistical analysis and to a large standard deviation. In addition, the number of samples included in the statistics is not very large at 77 or 80 samples. According to ISO 17994 (equation shown in ISO 17994 in section 5.4.3) 200 additional samples are necessary to obtain a statistically unambiguous result for the comparison of Legiolert max. and ISO *Legionella* spp. results. Considering Legiolert best and ISO *Legionella* spp. results, more than 2200 samples would be needed. This number is unrealistic and justified by the facts mentioned above.

An attempt was made to reduce the standard deviation in the ISO 17994 evaluation. Comparing results that showed a lower measurement uncertainty (exclusion of results smaller than three positive wells or cfu), could confirm statistically that Legiolert max. results, i.e. the higher value from 1 and 10 mL, were significantly higher than the *Legionella* spp. ISO results (see Table 14 e). In this context, the 1 mL Legiolert and 1 mL heat ISO results were also compared to exclude the influence of different volumes and extrapolation factors (see Table 14 g, h). By comparing the 1 mL Legiolert results with the 1 mL results of the ISO heat approach that yielded the final result, the standard deviation was clearly reduced (see Table 14 g). At almost 80 %, it corresponded to the normal value specified in ISO 17994. Due to the small data set (n = 17) statistically definite results were not obtained. The location of the “confidence interval” allowed

the assumption that the methods are equal (see Figure 11 on page 60). At the same time, the hypothesis was supported that the influence of the different volumes coupled with the different conversion factors has a major impact on the statistical evaluation. The comparison of 1 mL Legiolert with 1 mL heat ISO results (see Table 14h), which did not necessarily provide the final result, is negligible as the consideration would be too much in favour of Legiolert.

An average false-positive rate of 3.9 % was determined for Legiolert in this study. In addition, it should be noted that scrubber samples, which were contaminated with a high level of interfering bacteria, might show a higher false-positive rate. The use of only 1 mL sample with a longer pretreatment time can help in these samples. Data were obtained in further separate testing of scrubber waters indicating a better performance by using 1 mL (data not shown).

Legiolert is very suitable for the detection of *L. pneumophila* in cooling tower water. Applying the two test volumes of 10 and 1 mL, *L. pneumophila* concentrations from 10 to over 200,000 mpn/100 mL are determinable. With the UBA recommended procedures of the ISO method the lower limit of quantification is 5 cfu/100 mL, the upper limit of quantification is max. 300,000 cfu/100 mL.

L. pneumophila is very fastidious in terms of cultivation conditions and it must always be taken into account that more bacteria are present than can be recovered. For reasons of public health protection, the higher result of the two Legiolert volumes should therefore always be selected when reporting the result, even if the more uncertain value is chosen. This procedure would correspond to the recommendation of the German Federal Environment Agency (UBA) for the reporting of *Legionella* results in drinking water samples (154).

The ISO procedures were modified when comparing the 40 cooling water samples over a period of ten months. The modification was made in order to improve the method within the laboratory. The data set was quite small with n = 32. Thus, a statistically definite result was not obtained (see Table 50 on page 153). The location of the “confidence interval” of the comparison of Legiolert max. and *L. pneumophila*-ISO results (see Figure 61 a on page 153) suggested that Legiolert would yield significantly higher results if more samples were examined.

For the cooling tower operator, the comparison of the value determined in the cooling water sample with the test and action values of the German 42nd BImSchV determines which maintenance actions are to implement. In the first part of this study, Legiolert and the exact ISO approaches recommended by UBA (155) were compared. 60 to 75 % of the results were assigned to the same test or action value, depending on which results were considered (max. or best Legiolert results and ISO *L. pneumophila* or *Legionella* spp. counts, see Table 15 to Table 18 on page 61). Usually, statistically significantly more samples were assigned to a higher test

or action value with Legiolert. No statistical difference was confirmed for Legiolert best and ISO *Legionella* spp. paired data.

The results from the second part included 40 cooling water samples tested with modified UBA recommended ISO procedures and Legiolert (1 and 10 mL) over the ten-month period. In 75 to 77.5 % the same test or action values were exceeded by both methods (see Table 51 to Table 54 on page 154). Only when considering the Legiolert max. and the ISO *L. pneumophila* results the exceedance of test or action values was statistically significantly higher for Legiolert. When comparing the other data sets, the exceeding of higher test or action values with one method was not statistically significant.

The measurement uncertainties of the results of both methods are shown in Figure 62 on page 155. With the ISO method, more results with a low measurement uncertainty were obtained. About three quarters of the ISO results and about half of the Legiolert results were influenced by a low measurement uncertainty. The proportion of results influenced by a very high measurement uncertainty is 8 % (*Legionella* spp.) and 10 % (*L. pneumophila*) for the ISO method, 10 % for Legiolert best results and 17 % for Legiolert max. results. The fact that more results with a low measurement uncertainty were obtained by the ISO method may be due to samples with low *Legionella* concentrations, respectively colony counts slightly above ten, which were determined with the 20 mL test volume procedures of the ISO method. Tested by Legiolert, these samples will most likely range between four and nine positive wells (high measurement uncertainty) at 10 mL and between one and three positive wells (very high measurement uncertainty) at 1 mL caused by the smaller test volumes.

For Legiolert, results above test value 1 of the 42nd BImSchV (> 100 cfu/100 mL) from 10 mL test volume are associated with a low measurement uncertainty. From 1 mL the results are influenced by a very high or high measurement uncertainty depending on the number of positive wells. Results exceeding test value 2 (> 1,000 cfu/100 mL) or the action value (> 10,000 cfu/100 mL) of the 42nd BImSchV are influenced by a low measurement uncertainty with both 1 and 10 mL test volumes (see Table 59).

In order to know from which measurement uncertainty final results of the ISO method are influenced, the origin of the procedure providing the final result must be known. If the test value 1 of 42nd BImSchV is exceeded, results from 20 mL test volume procedures provide reliable results as described in Table 59. However, only results up to 400 cfu/100 mL may be reported (this corresponds to 80 cfu grown on the filter according to ISO). It should also be noted that in this study the filters of the 20 mL procedures were often not evaluable, because they were overgrown by interfering microorganisms.

Table 59: Measurement uncertainty of final results exceeding test and action values of the 42nd BImSchV, depending on the test volume.

Red: very high MU, 1 – 3 cfu or positive wells; orange: high MU, 4 – 9 cfu or positive wells; green: high MU, ≥ 9 cfu or positive wells. Upper quantification limit (UQL) of 20 mL test volume amounts to 400 cfu/100 mL. Grey fields: volumes are not able to cover the test or action value.

42nd BImSchV values	ISO [mL]			Legiolert [mL]	
	0.1	1	20	1	10
Test value 1 (> 100/100 mL)			UQL!		
Test value 2 (> 1,000/100 mL)					
Action value (> 10,000/100 mL)					

If test value 1 of 42nd BImSchV is exceeded, results from the 1 mL test volume procedure (heat treatment) - just like the 1 mL Legiolert results - are influenced by a high or very high measurement uncertainty. If test value 2 of 42nd BImSchV is exceeded, results from 1 mL procedures are influenced by a low measurement uncertainty, and those from 0.1 mL procedures by a very high or high measurement uncertainty. Results that exceed the action value are always influenced by a low measurement uncertainty.

4.1.2 Applicability of Pseudalert™/Quanti-Tray 2000 ® for the detection of *P. aeruginosa* in cooling tower water

The ISO reference method for the detection of *P. aeruginosa* in cooling water samples is based on a membrane filtration method and placing the filter on an agar plate. According to ISO 8199, the total number of colonies grown on the filter should not exceed 80 cfu and the number of target organism colonies should exceed 10 cfu. The maximum number of detectable colonies may be higher or lower, depending on how the filter is covered. The maximum evaluable colony count should be determined by verification tests. Cooling water samples are often contaminated with a high number microorganisms, which also appear on the membrane filter on Cetrimide agar and may inhibit the growth of *P. aeruginosa*. By dilution series of each sample, the influence of the interfering non-target microorganisms could be reduced. However, this would also involve an enormous amount of work. An advantageous alternative is the most probable number method Pseudalert™/Quanti-Tray 2000® from IDEXX. We could show in a comparative study (142) the advantages of Pseudalert that become apparent in:

- a significant improvement of the enumeration of *P. aeruginosa* in industrial samples,
- a better performance,
- a more than tenfold higher upper quantification limit using Quanti-Tray2000,
- a shorter incubation time,
- the omission of the toxic Nessler's reagent,
- the lack of confirmation steps.

With ISO 16266 Part 2 most probable number methods are authorized for the detection of *P. aeeruginosa* in water samples (82). In Germany, Pseudalert/Quanti-Tray is already a recognized method for testing *P. aeruginosa* in drinking water according to paragraph 15, section 1 of the Drinking Water Ordinance.

Considering the position of the confidence interval (see Table 56 and Figure 64 on page 157), it is reasonable to assume that if more samples had been examined, both methods could have given equal results for the detection of *P. aeruginosa* in these cooling tower water samples.

4.1.3 Applicability of the ISO method for the detection of heterotrophic plate counts in cooling tower water

According to the WHO, the detection of HPC serves to verify the effectiveness of biocide treatment (42). As described in section 3.2.3, the ISO method leaves much room for interpretation of heterotrophic plate counts in cooling water. There is no exact specification when reading the plates (with regard to the explicit test volume, the use of a counting grid and magnification level).

Experience has shown that the detection of heterotrophic plate counts in industrial water samples with the ISO method (73) is influenced by the following issues:

- the counting of plates is very individual, depending on the eyesight of the examiner and the nature of the colonies (partially very pale / very small / confusion with other particles in the sample / multicellular organisms possibly leading to erroneously multiple counts),
- the counting limits of ISO 8199 (81) are often exceeded when using a counting grid,
- the results are influenced by a very high standard deviation (90),
- in general the distribution of microorganisms in solutions and especially in cooling tower water is not ideal, so that the use of a small sample volume is accompanied by a high measurement uncertainty,
- a very small volume is applied to evaluate the microbial situation of an entire cooling tower.

Thus, it is questionable whether the ISO method is suitable for the detection of HPC in industrial water samples. The ISO method urgently needs to be revised regarding test volume, performance of dilutions (number and dilution steps), counting mode (with or without optical zoom or counting grid) to harmonize the procedures during HPC detection to produce better comparable inter-laboratory results.

The VDI Cooling Tower Code of Practice 2047-2:2015 proposes the additional performance of dip slide tests to control HPC by the cooling tower operator (159). Dip slide tests are generally

used to control microbiological growth processes. In the dip slide test, the culture medium is immersed directly in the cooling water and then incubated. With the help of a reading mask, the bacteria concentration in powers of ten per millilitre can be estimated (22). The cooling tower operator can carry out the dip slide test more frequently and more cheaply than the laboratory test with minimum effort and thus detect concentration fluctuations more quickly. Due to the larger sample volume, the easy and quick handling and the simpler reading of the result, the Dip slide test seems to be more suitable for HPC monitoring in cooling water than the ISO method in the sense of the 42nd BImSchV.

The current reference value determination according to 42nd BImSchV includes the first six values of the measurement series. The reference values are then fixed and neither adjusted to following decreasing nor increasing fluctuations. Therefore, it seems reasonable to calculate the reference values more dynamically by including new values in the reference value determination. A requirement for including new values in the reference value calculation is that the current reference values have not been exceeded. For example, reference value determination by forming the mean value, which is always updated, or a moving average over a certain number of measurements could be used for this purpose.

The HPC method has its origin in the drinking water analysis. The two incubation temperatures were established to detect environmental contamination (22 °C) or faecal contamination of the water and potential pathogens (36 °C) (172). Except for the aerosol transmission of *Legionella*, no public-health relevant distribution of microorganisms from cooling towers is expected. During cleaning and maintenance actions, eye, skin and ear infections are possible due to contact with *P. aeruginosa*. The personal protective equipment, which has to be worn during such work, should inhibit these infections (159). This bacterium can be detected selectively. The observation of biofilm formation in the system should also be possible when only one incubation temperature is applied. Thus, it was examined, whether the HPC of the two incubation temperatures differed statistically significant by applying the ISO 17994 relative difference approach (79). For the 17994 analysis, paired results were excluded which lay below the detection limit at both incubation temperatures and which at least at one incubation temperature exceeded the upper quantification limit or were not evaluable.

The ISO 17994 outcomes, which include zero counts at one incubation temperature while the other temperature was quantifiable are shown in Table 34 a) to c) on page 97, indicated significantly higher results for the incubation at 36 °C considering the usual 20 % as a permissible difference limit. Due to the generally high dispersion of HPC (90), an extension of the difference limits is proposed. Setting the difference limit at 30 %, the ISO outcomes indicated for the "Total" and "42nd BImSchV" data sets that the statistical difference was indifferent. For the “other

industrial sites" data set the difference remained significantly. It should be noted that the samples of the other industrial sites were taken from a few different locations where normally warm process water temperatures were recorded probably resulting in the presence of mesophilic species.

These analyses in Table 32 a) to c) on page 94 reflected slightly biased results. At 36 °C, quantifiable results were obtained much more frequently, which were below the detection limit at 22 °C, as vice versa. These paired data were included in the ISO 17994 analysis. In contrast, the substantial proportion of results that were quantifiable at 22 °C but exceeded the upper quantification limit or were not evaluable at 36 °C were not included in the analysis. To reduce the bias only quantifiable paired data were analysed according to ISO 17994 as shown in Table 34 d) and e). For the "42nd BImSchV" data set the ISO 17994 outcome was indifferent (see Table 34 e). The "Total" data set exceeded the difference limit by 0.8 % and should therefore also be accepted as "indifferent" (see Table 34 d).

An "indifferent" outcome signals that no statistical difference was confirmed and that the methods are practical equal (79). With the outcomes of the ISO 17994 analysis, the first impression is that incubation at 36 °C is advantageous.

Of the 145 ("Total" data set) / 141 ("42nd BImSchV" data set) 36 °C quantifiable and 22 °C zero counts paired data, 103 ("Total") / 101 ("42nd BImSchV") counts were below 100 cfu/mL. Only five counts each exceeded 10,000 cfu/mL. Of the 60 ("Total") / 59 ("42nd BImSchV") paired data that were quantifiable at 22 °C and above the quantification limit or not evaluable at 36 °C, 28 ("Total") / 27 ("42nd BImSchV") counts were recorded above 10,000 cfu/mL. Thus, each incubation temperature has its advantages. While plates incubated at 22 °C are more frequently quantifiable for highly contaminated samples, incubation at 36 °C is accompanied by a higher recovery and a shorter incubation period.

The fact that the results are higher for 36 °C can probably be explained by the fact that, in general, the proportion of mesophilic microorganisms in the cooling tower is higher than that of psychrophilic ones.

In summary, based on the results, incubation at one temperature should be sufficient to ensure good monitoring. It seems reasonable to choose the incubation temperature cooling tower individually. Thus, the incubation temperature could be chosen according to how high the average cooling water temperature is and at which incubation temperature the most quantifiable results are obtained. Choosing the incubation temperature close to the average water temperature would well represent the general microorganisms of the cooling tower able to grow under the nutrient and incubation conditions.

4.2 Assessment of the retrospective analyses for the prediction of legionellae concentrations and heterotrophic plate counts

The retrospective process parameters and microbial data analyses were performed to create a tool for the prediction of HPC and *Legionellae* with the aim to introduce a process parameter-based risk assessment for the different cooling towers.

In general, the typically recorded process parameters pH level, temperature, total hardness and turbidity are possibly associated with legionellae growth (3). In the study of Armero et al. these parameters were used to generate a complex system of a probabilistic engine within a master–slave architecture to predict the risk of legionellae in cooling systems (4). Unfortunately, it has not been reported whether the system has proven to be effective in preventing increased concentrations or outbreaks.

At the beginning of the retrospective study, redox potential, water temperature, pH, conductivity and DOC values were considered as possible parameters for the regression analysis. Because DOC, conductivity and pH values were only subject to very small fluctuations in the observed cooling towers (data not shown) no regression analyses were performed for these process parameters, but only for redox potential and water temperature data. The best correlations of the different analyses mostly indicated a medium correlation. For each parameter the regression lines were plotted, which showed the highest correlation above 0.3. In some cases, however, such low correlations were found that no regression lines were generated due to the weak statistically significant correlation. Of course, the prediction tool cannot replace microbiological monitoring and surveillance. But with the help of the prediction tool, the cooling tower operator can be alerted earlier and react to possible changes in the microbiological condition of the cooling system in the event of certain changes in the process parameters (e.g. rapid increase or decrease in redox potentials per time unit, increase in water temperature). Actions of the operator like checking the cooling system, laboratory tests and, if necessary, additional biocide dosing can be initiated at an early stage. The quality of the prediction depends on the nature of the regression line. As much data as possible should be included in the creation of the regression line. The data points should evenly flank the regression line. Well applicable regression lines of this study are those of the right chart of Figure 23 and the left and right chart of Figure 24 on pages 76 and 77. Regression lines whose course is mainly influenced by very few values (e.g. right chart of Figure 17 on page 70, left chart of Figure 23 on page 76, middle chart of Figure 24 on page 77) or which have large free gaps along the course (mid chart of Figure 34 on page 87) are not suitable as prediction tools, even if they show a good Pearson correlation. To what extent the regression line can be used to quantify the current process parameter value depends on the ordinate range covered by the regression

line. The left chart in Figure 24 for the prediction of legionella values in Cooling Tower 2 offers a wide ordinate range for the determination of legionellae concentrations from 10 to 10^4 cfu/100 mL, so that concentrations can be estimated with the current process parameter value. The regression line in the right chart of Figure 23 for the prediction of HPC at 36 °C, extends only over a narrow ordinate range of 1.5 log steps and thus, if it all, can only allow qualitative statements about the increase or decrease of HPC.

As mentioned above, no analyses were performed for DOC, pH and conductivity values. It is conceivable that in other cooling towers, prediction tools based on these process parameters are feasible.

In preliminary tests, the retrospective analyses conducted in this study were also carried out without outliers. The regression lines showed the same trend (slope) as those including outliers. However, the correlation was always weaker than with outliers. For this reason, only the regression analyses with outliers were shown. It is quite conceivable that in other cooling systems the inclusion of outliers could provide a weaker correlation or even change the slope. The latter would have a great influence on the interpretation of the correlation of the parameters. Therefore, regression analyses must always be checked for their meaningfulness.

Regression lines, which were created from the combination of retrospective microbiological data and modified process parameter data, can be used as prediction tools, if

- the data set is sufficiently large,
- the regression line has at least a medium correlation (> 0.3),
- the regression line is evenly flanked by data points and
- the covered ordinate range is sufficiently large.

It is conceivable that there are cooling towers where no prediction tool can be created based on process parameters. It should be mentioned again that prediction tools are not able to replace microbiological monitoring and surveillance by laboratory tests, but serve as a proactive risk assessment tool to detect a possible increase of potentially pathogenic bacteria at an early stage for the protection of public health.

4.3 Microbiological planktonic concentrations and biofilm formation in the four cooling towers

The cooling towers investigated in this study were (and are) all subject to good monitoring of both process and the microbiological parameters *Legionella* and HPC. They are located very closely (max. 2 km linear distance), and thus, subject to the same climate conditions. All cooling

towers in this study are fed by the same make-up water. These conditions can be considered the same, too. The obvious differences lie in the construction (building material, volume) and the biocide treatment. Presumably, the pipe network and the number and type of heat exchange processes have an influence on the biofilm formation. Furthermore, the nutrient input, e. g. from surrounding trees due to leaf or pollen intake, will influence the biofilm formation. However, these variables could not be determined and remain unknown. In this study, the biofilm formation in areas with high flow in the cooling water basin was investigated.

4.3.1 Similarities and differences of the four investigated cooling towers

During the ten-month observation period in Cooling Tower 1 very low concentrations of HPC, *Legionella* and *P. aeruginosa* were recorded in the water samples and on the plates as shown in Figure 41 on page 104. Even during the deactivated biocide treatment, the three parameters in the water samples increased only slightly. HPC and *P. aeruginosa* were detectable in increased concentrations in the cooling water after reactivation of the biocide treatment by peracetic acid shock dosing due to biofilm detachment, but were considerably reduced within one week by the reactivated chlorine dioxide treatment. There was no *Legionella* peak after the peracetic acid treatment, which suggests that even the few ClO_2 doses (traceable in Figure 66) in combination with the peracetic acid treatment had a biocidal effect on *Legionella* as shown in Figure 67. HPC were always detected in the biofilms on the Cooling Tower 1-plates within the ten-month investigation period. Apparently and statistically (Mann-Whitney-U-test) there was no difference between the short and long interval (see Table 60). *Legionella* was sporadically detected on both types of plates during the observation period. *P. aeruginosa* was found more abundantly on the short-term plates during the summer months. The impression was created that higher counts or concentrations were generally found on the short-term plates. Statistically, however, no significant difference between short- and long-term interval was confirmed by the Mann-Whitney-U-test as shown in Table 60. It should be emphasised once again that statistical tests are not very reliable due to the small data sets. Nevertheless, statistics were performed to possibly recognize a trend. With the reactivation of the biocide treatment, a rapid decrease of planktonic and sessile microorganisms was expected. Although strong algae growth was recorded, the effect on the three investigated parameters provided no reasons for concern according to the requirements of the 42nd BImSchV. It is assumed that unconsumed chlorine dioxide or O_2 radicals were still present in sufficient concentrations to limit biofilm formation.

Chlorine dioxide can limit biofilm formation on surfaces (137). This could be the reason for the higher concentrations on the short-term plates, as chlorine dioxide covered the surface of the long-term plates and prevented successful (re-)attachment of planktonic bacteria. Due to the

peak of planktonic HPC after reactivation of the biocide treatment shown in Figure 67, it is suspected that an increased biofilm formation has occurred in other cooling tower areas than on the plates in the cooling tower basin. The high shear forces of the strong flow rate in the cooling tower basin probably had a limiting effect on the biofilm formation (115, 137). After reactivation of the biocide treatment, the numerous algae grown on the cooling tower internals apparently died and fell into the water of the cooling tower basin. The increase in nutrients caused by dead biomass initiated into the cooling water (137) was probably the reason why HPC was still higher for weeks after reactivation of the biocide treatment than before deactivation. It is clear that in the selection of biocides, both decomposition and killing properties must be combined in order to avoid rapid regrowth through the nutrients provided by the dead biomass (137). The effects of a failure in biocide treatment in a more hazardous, i. e. *Legionella*-containing system could therefore be devastating in a short time and lead to an outbreak of legionellosis.

Cooling Tower 2 was characterised by the tough, red-brown to black biofilms formed on the plates and by the presence of snails and small crustaceans. The water samples showed fluctuating curves especially for HPC. On the long-term plates, higher values were detected for HPC after half of the observation period than on short-term plates (see Figure 44). The difference in concentration between long- and short-term polyethylene plates was found to be statistically significant as shown in Table 60. *Legionella* were repeatedly detected in fluctuating concentrations in water samples, but only rarely on the plates. *P. aeruginosa* occurred frequently in the water samples. The bacterium was detected on the short-term plates during the summer months. A statistically significant difference was not verified for the concentrations of long-term and short-term plates (see Table 60).

The fluffy, soft, thick, algae containing biofilm characterized the plates of Cooling Tower 3. On the long-term plates, higher values were detected for HPC after half of the observation period than on short-term plates. The concentration differences of short- and long-term plates were statistically significant for HPC at 22 °C on stainless steel and for HPC at 36 °C on polyethylene (see Table 60). The regular *Legionella* and *P. aeruginosa* findings both in the water samples and on all plates also characterized the Cooling Tower 3. A statistically significant difference in concentration of short- and long-term stainless steel plates was recorded for *Legionella* (see Table 60). Cooling Tower 3 had the highest *Legionella* species diversity as listed in Table 42.

The invisible or very thin biofilms grown on the plates of Cooling Tower 4 led to the assumption that due to the visual similarity of the plates comparable concentrations to those in Cooling Tower 1 would be found. The few algae on the cooling tower internals also suggested that

inconspicuous microbial concentrations were to expect. The continuous and partly high concentrations of *Legionella* and *P. aeruginosa* in the water samples and on the plates as shown in Figure 50 refuted these assumptions. There were no noticeable or statistically significant concentration differences between short- and long-term plates. Whether areas with thicker biofilms were present in the cooling system and whether only the initial attachment state was recorded on the examined plates in the cooling tower cannot be excluded. Since in oligotrophic environments mature biofilms may consist of little more than a sparse covering of cells with relatively little structural complexity (144), under the stress conditions in the cooling tower (laminar and turbulent flow, biocide treatment), a low biofilm thickness is conceivable in all areas. It can definitely be concluded that a low biofilm thickness is not associated with a low risk of health-related microorganisms. However, a low biofilm thickness is undoubtedly desirable for the functioning of the cooling tower due to the efficiency of the heat transfer and the protection of the materials (11, 53, 137).

It was of interest to check whether statistically significant microbial concentration differences could be verified for the two materials. As mentioned in section 3.3.2, the values on the both plate materials did not differ significantly considering the same investigation interval regarding each cooling tower separately. The combined data sets of short- and long-term values for each cooling tower and parameter also did not show any statistically significant difference for both materials (SS SI+LI vs. PE SI+LI; Mann-Whitney-U-test, two-sided observation, significance level 5 %). The result is consistent with the heterogeneous findings of other studies, in which the same or a higher biofilm formation was observed for both plastic and stainless steel surfaces (86, 108, 118, 132, 174).

Subsequently, the two study intervals were compared (short interval vs. long interval). The results are shown in Table 60 and already described above in section 3.3.4.

The examined parameters in Cooling Towers 1 and 4 showed no significant differences in concentration for the two examination intervals; neither for the plate material specific data sets (SS_SI vs. SS_LI, PE_SI vs. PE_LI) nor for the combined data sets (SS+PE_SI vs. SS+PE_LI). Only very thin biofilms were recorded on the plates of these two cooling towers. This indicated that the biocide treatment conditions were able to control the biofilm formation in such a way that a further biofilm development was not possible.

Table 60: Statistical significant differences of growth on short-term and long-term plates for each cooling tower.

PE: polyethylene; SS: stainless steel; yes: significant difference of growth on short-term and long-term plates; no: no significant difference of growth on short- and long-term plates; not done: no statistical analysis performed due to lack of quantifiable results. Statistical calculation was performed with the Mann-Whitney-U-test using a two-tailed hypothesis and a significance level at 5 %.

CT	Plate type	HPC 22 °C	HPC 36 °C	*Legionella* spp.	*P. aeruginosa*
1	SS	no	no	not done	not done
	PE	no	no	not done	not done
	SS+PE	no	no	no	no
2	SS	no	no	not done	not done
	PE	yes	yes	not done	not done
	SS+PE	yes	yes	no	yes
3	SS	yes	no	yes	no
	PE	no	yes	no	no
	SS+PE	yes	yes	yes	no
4	SS	no	no	no	no
	PE	no	no	no	no
	SS+PE	no	no	no	no

While *Legionella* and *P. aeruginosa* were detected on almost all plates of Cooling Tower 4, *Legionella* occurred rarely on the plates of Cooling Tower 1. *P. aeruginosa* was more frequently detected in these two cooling towers on the short-term plates of both materials, although not statistically significant. The bacterium is known for its ability of initial attachment to surfaces (111, 144). The reduced - but statistical not significant – occurrence of *P. aeruginosa* on the long-term plates of these both cooling towers indicated that a certain maturation of the biofilm resulted in its replacing by other microorganisms as also observed e. g. by Liu et al. (94).

In Cooling Towers 2 and 3, significant differences were recorded for the parameters on the short-term and long-term plates as shown in Table 60. In both cooling towers HPC of both incubation temperatures were found in higher concentrations on the long-term plates. In Cooling Tower 2, statistically significant higher concentrations of *P. aeruginosa* were recorded on the short-term plates. In Cooling Tower 3, statistically significant higher *Legionella* concentrations were observed on the long-term plates. In Cooling Tower 2, the pattern of occurrence of *P. aeruginosa* corresponded most closely to the expectations of its ability to colonize surfaces (111, 144). Low concentrations of the bacterium were detected in the cooling water. On the short-term plates, partly high concentrations were detected. The bacterium was rarely detectable on the long-term plates, as it was probably displaced from the biofilm by secondary attached microorganisms (144). High cell densities in biofilms promote intense competition for nutrients and other resources as well as progressive deterioration of conditions due to depletion of resources and accumulation of metabolic wastes and this may force some microorganisms into inactive states or even kill them (20). The occurrence of *P. aeruginosa* in Cooling

Tower 3 is probably not exclusively attributed to its ability to colonise surfaces, but to biofilm conditions that have promoted the persistence of the bacterium.

For each cooling tower, characteristic colony morphology types were recorded on the yeast extract agar plates, which could be clearly assigned to the respective cooling tower. These specific colony morphology types were registered on the HPC agar plates of the water samples, the short-term and long-term plates of both materials, indicating that all regularly planktonic detectable microorganisms are anchored in the biofilm. This hypothesis is supported by the fact that exclusively planktonic-living microorganisms cannot persist in the cooling tower, as they would be removed from the system by evaporation and blowdown (94, 146). The facts that no significant microbial concentration differences were recorded on the two plates materials and that characteristic colony morphologies occur in the cooling towers suggest that the biocide has a greater impact on biofilm formation than the construction material. The observation that a large proportion of colony morphology types were recorded on both short-term and long-term plates suggested that the microbial community formed on the short-term plates persisted for largely the entire observation period. This observation is confirmed by findings from Liu et al. (94). The colony morphology types of the short-term plates detected in Cooling Towers 1 and 4 using the HPC method mainly corresponded to those of the long-term plates, while on the long-term plates of Cooling Towers 2 and 3 further colony morphologies were recorded regularly. As described above, none of the parameters of the short-term and long-term plates in Cooling Towers 1 and 4 differed statistically significant. These facts suggest that the biocide treatment was able to keep the biofilms in Cooling Towers 1 and 4 mainly in the stages of initial attachment and early biofilm formation. Presumably, the majority of the biofilm consisted of microorganisms involved in the first colonization. The fact that further colony morphology types were frequently detected on the long-term plates of Cooling Towers 2 and 3, and that HPC and *Legionella* or *P. aeruginosa* differed significantly for the short- and long-term interval, indicated that the biocide treatment was not able to regulate biofilm formation sufficiently, so that maturation of the biofilm took place. At this stage, in addition to the first colonizers, other microorganisms were able to colonize the biofilm (144).

Based on the results of the biofilm formation experiments, it is assumed that biocides in general can control biofilm formation in the status of initial attachment and early biofilm formation up to a threshold value of biofilm thickness. Above this certain thickness, a maturation of the biofilm seems to be possible as occurred in Cooling Towers 2 and 3. Mature biofilms exhibit an increased resistance to killing by biocides (92, 137). The reason for the increased resistance seems to be the three-dimensional structure and the EPS (158). Another reason might be the presence of “persister cells” that have been described as special phenotypes in biofilms able

to survive to an increased degree under biocide and other stress conditions, and influencing increased biofilm formation under less stress conditions. (137). Therefore, they can probably synergistically lead to increased biofilm formation when the biofilm has reached a certain thickness, thus better protecting the “persister cells” and stimulating their proliferation. During the maturation process the composition of the microbial community shaped by the specific conditions in the cooling tower changes (119, 149). Furthermore, the biofilm composition varies over the cooling tower system influenced by different temperatures, materials and local habitats (94, 121). Thus, the biocide used should have biofilm formation limiting properties in addition to the killing properties (137). In this research project, these combined properties were attributed to chlorine dioxide.

4.3.2 Seasonal microbiological dynamics

Seasonal dynamics with increased planktonic HPC and *Legionella* concentrations during summer/autumn were observed regarding the data from the retrospective study (see section 3.2.2, page 90). For Cooling Towers 2 and 4 the difference of HPC in cold and warm months was statistically significant. The *Legionella* concentrations of cold and warm months differed significantly in Cooling Tower 2 within the seven-year observation period. In the biofilm experiments, during the warmer months higher concentrations were recorded on the plates in some cases. In the water samples, seasonality was observed for HPC in Cooling Towers 1 and 2 and for *Legionella* in Cooling Tower 4. During the first water sample investigations in February, all cooling towers showed comparable high HPC. These high counts were probably an artefact due to the processing of HPC the next day and not the same day as performed for the other sampling dates.

The literature reflects a heterogeneous picture of the seasonality of microbiological parameters. Partially studies showed no seasonal dynamics of *Legionella* in cooling towers (88, 125, 152). In another study the microbial community composition was highly dynamic and subject to seasonal change (149). Abiotic factors like seasonal changes in temperature or operational–technical parameters having impact on microbial growth were responsible for seasonal patterns in a hospital cooling tower (88). Systems operated year round showed relatively constant numbers of planktonic and sessile *L. pneumophila* (67). In contrast high viable counts of *L. pneumophila* were found in continuously operated cooling towers in the winter period (152).

The four investigated cooling towers reflect the heterogeneous picture of the literature, although they show many similarities in terms of location, make-up water, general mode of operation. In Cooling Towers 1 and 3, regarding the retrospective data pretty constant *Legionella* concentrations and HPC were recorded, although the two towers are in fact very different in

their microbial composition. In Cooling Towers 2 and 4, significant seasonal fluctuations were recorded during cold and warm months .

In summary, seasonal dynamics are cooling tower specific and probably depend in particular on abiotic factors such as increased water and air temperature and nutrient input from make-up water and ambient air. Nevertheless, *Legionella* tended to increase in late summer/autumn and HPC in midsummer.

4.3.3 Influence of the make-up water

In other studies, partly by using 16S rRNA analyses, was shown that the make-up water has a great influence on the microbial community in the cooling tower (23, 94, 117). In this present study, no 16S rRNA analyses were performed that would have allowed a total comparison of microbial composition on the phyla and genus level. Results of 16S rRNA analyses in cooling tower water samples indicated that, in general, Proteobacteria (Sphingomonadaceae, Comamonadaceae, Hyphomicrobiaceae) made up the major part of the cooling tower water microbial flora (23, 95, 117, 119). Cyanobacteria, Bacteroidetes and Actinobacteria were also detected in nominal quantities. Particularly at the genus level, the cooling tower water composition was very similar with small fluctuations depending on the location, i.e. the make-up water (117). Hence, the fact that the HPC method was able to show cooling tower specific colony morphologies seems astonishing, as the method only detects about 1 % of all present microorganisms (43). Individual cooling tower conditions (local habitats, temperature, material composition, flow rates, chemical water composition) cause individual microbiomes for each cooling tower (94, 119). The investigated cooling towers of this study mainly differ in biocide treatment and construction and show cooling tower specific colony morphology types detected with the HPC method. Since the microbiological concentrations and colony morphology types did not differ on the two plates materials in each cooling tower, not the construction material but the biocide used seems to mainly cause the specific colony morphology types and thus possibly a cooling tower-specific microbiome.

4.3.4 *Legionella* in biofilms and cooling tower risk management

Legionellae were detected in all four cooling towers. Consequently, the bacteria found conditions in all cooling towers that enabled their survival. Thus, each of the investigated cooling towers might bear the risk of causing legionellosis under certain conditions. It is difficult to determine which condition promotes or inhibits *Legionella* survival in cooling towers, since the exact interactions between bacteria, protozoan and human hosts and environmental conditions (particularly in the aquatic reservoir) leading to a legionellosis outbreak remain mainly unknown

(41, 112). The complex relationships between nutrient availability and metabolism, quorum sensing, predation, temperature, aeration, flow rate and surface material determine the biofilm composition and the phenotypic characteristics of the microorganisms (94, 119, 137, 146, 147). The biofilm composition has an influence on the occurrence of legionellae in biofilms. *Legionella* can persist in the biofilm even without the presence of host cells, but their reproduction takes place mainly in host cells (40, 98, 110). Free living *L. pneumophila* are able to attach to surfaces, to form dense clusters, but not to form robust biofilms (98, 147). The bacterium competes with other strains for biofilm colonization (98). Thus, the presence and ability to multiply in the cooling tower will depend on the presence of host cells and other synergistic microorganisms and/or the successful colonization of biofilms, or on the absence of antagonistic biofilm colonizers that displace free *Legionella*. It is assumed that the phenotypic expression determines whether *Legionella* can persist freely in the biofilm. Pereira et al. showed that *Legionella* strains represented a core community in a cooling tower, emphasizing the importance of cooling towers as a substantial environmental reservoir (119). The presence of biofilms causes damages in the cooling system, reduces the heat transfer, decreases the energy deficiency and protects possible pathogens (11, 110). As discussed in 4.3.1, the thickness of the biofilm does not correlate with the (planktonic or sessile) *Legionella* spp. concentration. The circulating water is seeded by the biofilm and aerosolized by evaporation in the heat transfer process possibly resulting in transmission of pathogens (9, 42, 94, 95, 119, 149, 159). Thus, preventing the formation and maturation of biofilms in evaporative cooling systems should have the first priority. Biofilm thickness can be determined the easiest and cheapest way by inspecting cooling tower internals and coupons, which are placed at different locations in the system in the best case. Impedimetric biosensor monitoring represents another, more expensive method, which has not yet been sufficiently validated (93, 122).

In this study, Cooling Tower 1 continuously treated with chlorine dioxide showed low concentrations of the tested parameters both in the water samples and on the plates. In combination with the results of the retrospective analysis and the risk factor calculation, a low risk of causing legionellosis outbreaks is assigned to this cooling tower. The microbial concentrations particularly for *Legionella* spp. of the ozone treated Cooling Tower 2 seemed to be clearly lower than in the previous years included in the retrospective analyses, where the other cooling tower of the system was monitored in routine testing. It is not clear whether the lower microbial concentrations are attributed to a change in the operating mode or the fact that the other cooling tower was sampled. However, since the same cooling system is observed, the risk assessment includes the retrospective data. Nevertheless, the risk factor calculation revealed a low risk to cause legionellosis outbreaks for Cooling Tower 2. The chlorine treated Cooling Tower 3 was assigned a low risk due to its retrospective *Legionella* concentrations. Chlorine is capable of

reliably killing bacteria (109, 117, 137). The microbial concentrations on the stainless steel and polyethylene plates and the biofilm formation on the cooling tower internals were not considered in the risk factor calculation, but might result under certain conditions in high planktonic concentrations increasing the risk causing legionellosis. The chlorine/sodium bromide treated Cooling Tower 4 displayed the highest risk of causing a legionellosis outbreak due to frequently high *Legionella* concentrations in the water samples and on the plates during the ten-month observation. The calculated risk factors for Cooling Tower 4 indicated that this cooling system should always be monitored especially thoroughly. Preventive peracetic acid shock dosing could be carried out preventively at regular intervals.

Both the data from the retrospective analysis and the biofilm study showed that *L. pneumophila* strains were most frequently found in the cooling towers. This may result from the fact that GVPC agar was used. GVPC agar is selective for *L. pneumophila* growth. It might also suggest that *L. pneumophila* are better adapted to the conditions in the four investigates cooling towers in particular, and perhaps even in general, than other *Legionella species*.

The simultaneous detection of *P. aeruginosa* and *L. pneumophila* on the short-term and long-term stainless steel and polyethylene plates in Cooling Towers 3 and 4 did not confirm the findings of Paranjape et al. The scientists postulated that high levels of continuous applied chlorine are accompanied with the presence of *Pseudomonas* and the lack of *Legionella* (117). Since the chlorine disinfection in Cooling Tower 3 and 4 was activated several times per day when the redox potential dropped below 400 mV, a continuous presence of chlorine can be assumed, even if the exact concentrations remained unknown. It is assumed that *Pseudomonas* and *Legionella* colonize the same niches in the biofilm or have an antagonistic effect (143). Accordingly, the two species might have colonized spatially separated locations on the investigated 25 cm^2 areas. Alternatively, depending on the prevailing environmental conditions or the presence or absence of other microorganisms, coexistence of the two species may or may not be possible individually for each habitat. If this assumption is correct, this might be true for many other microorganisms in biofilm communities.

The risk factor calculation shown in subsection 3.3.3.2 is obviously not sufficient for a complete risk assessment of the entire cooling tower. The biofilm formation in the cooling tower, for example, as well as many other influencing variables (temperature, construction, location...) are not considered. Nevertheless, the value is able to represent the planktonic state and development over a certain period. Moreover, the value is indicative of the minimum measures that should be taken in case of faulty operation (e.g. failure of (dosing) pumps, input of potential nutrients etc.). The period chosen for the risk assessment should neither include too much nor too little data, as the calculation is based on averaging. A too long period of time weakens less

frequently occurring high *Legionella* concentrations too much. A too short period might not be sufficiently representative. A period of three years, which should always be updated by the current measured value, is considered reasonable and shown in Figure 70. The last 40 results were chosen as the basis for calculation. In contrast to subsection 3.3.3.2, the last 40 HPC results were averaged instead of using the reference value.

The interval including 40 values represents the same cooling tower ranking of the risk of a high planktonic *Legionella* (and HPC) concentration possibly causing legionellosis. For Cooling Tower 1, the risk values did not differ regarding the three and nine-year periods. Hence, stable conditions prevailed in Cooling Tower 1. In Cooling Tower 2, the risk factors based on the calculation of the latest 40 values were higher than the nine-year calculation and considerably higher than the ten-month calculation.

Cooling Tower 2 was built in 2018 and belongs to a cooling system including two cooling towers. The water of the other cooling tower is routinely tested in the laboratory for Technical Hygiene at the IHPH. The results of the routinely monitoring, respectively of the other cooling tower, were chosen for the nine-year and 40-results calculation. The ten-month calculation was done for the results of Cooling Tower 2 obtained during the biofilm experiments. By now, it was assumed that planktonic concentrations might not differ markedly, because large quantities of water pass through the entire system. However, request to the operator revealed that problems with the system-central ozone pump had repeatedly occurred in the past that might have caused high planktonic *Legionella* and HPC concentrations resulting in these risk factors.

In Cooling Tower 3, the nine-year calculation differed rarely from the 40-values calculation, but the risk factors of the ten-months observation period are considerably higher. As with Cooling Tower 2, the cooling system of Cooling Tower 3 also consists of two cooling towers, of which the other is subject to routine monitoring. In this case, though, the cooling tower subject to monitoring shows the lower concentrations.

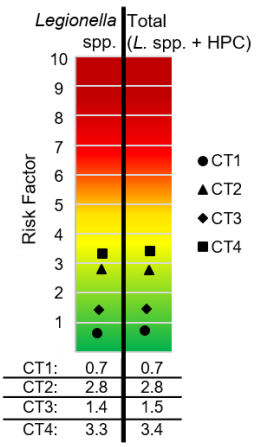


Figure 70: Risk factor calculation of the latest 40 values.

As mentioned above, the assumption seems to be valid for the risk factor calculation in Cooling Tower 4 that an excessively long observation period weakens high *Legionella* concentrations and that the risk factor is influenced too strongly during a very short period by occasionally high concentrations.

Based on the comparison of the risk factors of different cooling towers of the same system, it should be considered whether the other cooling tower units existing in the same cooling system should be monitored at least temporarily.

This risk assessment presented here certainly has the potential to act as a trend-setting element for the cooling tower operator in the prevention of high *Legionella* concentrations and thus legionellosis. It is an easy-to-use tool, and may also be applicable for the laboratory and monitoring authorities. The calculation performed in this work is based only on the *Legionella* concentrations and heterotrophic plate counts since the focus of interest was the cooling tower specific risk of causing a legionellosis outbreak and of an increased biofilm formation.

Since this risk assessment is based on the test and action values of 42nd BImSchV cooling towers with a risk factor smaller than three are in a very good operating mode harbouring a low risk of causing a legionellosis outbreak. Cooling towers with risk factors higher than three and below six should be monitored very thoroughly. In these cooling towers, small disturbances can possibly have a great influence on *Legionella* and biofilm formation.

The calculation may include further influencing parameters since the risk factor calculation can be easily adapted individually. To what extent further parameters (e.g. additional microbiological parameters *as P. aeruginosa*, water temperature, disturbances in the system, nutrient input) are able to influence the risk factor calculation and which risk values would be applicable should be tested thoroughly. Nevertheless, the focus should remain on the cooling tower-specific risk of causing a legionellosis outbreak. The introduction of additional parameters must not reduce the *Legionella* risk factor much. However, the calculation with the influence of further parameters seems to be realisable exclusively for the cooling tower operator, since the information exchange is probably very complicated.

Finally, the cooling water monitoring according to 42nd BImSchV and VDI 2047-2 (1, 159) is evaluated. According to the WHO, the detection of HPC serves to verify the effectiveness of biocide treatment (42). According to the 42nd BImSchV, the average value formed from six successive measurements serves as a reference value for the cooling system, and exceeding the reference value by a factor of 100 results in a complaint. VDI 2047-2 also contains control strategies for exceeding the HPC by a factor of 10 or 100, but it does not specify exactly which value is the reference. Both the 42nd BImSchV and VDI 2047-2 allow the value 10,000 cfu/mL

to be used as a reference value, as it is often used as a reference value for HPC (159). Cooling towers commonly show bacterial counts between 10^3 and 10^7 cfu/mL (8) and HPC account for about 1 % of the total population (43). Detection of HPC is not primarily intended to detect health-risk related microorganisms and does not correlate with *Legionella* concentrations (8, 27). *Legionella* persist in biofilms and an increase in biofilm formation, respectively the increase in specific (possibly non-culturable) microorganisms in the biofilm, could cause an increase in *Legionella*, but the exact relationships in the biofilm remain largely unknown (26, 69, 94, 95, 110, 117, 119, 149, 152, 165). If processes in the microbiome should be determined which are connected with the proliferation of *Legionella* and for which rapid detection methods are available, such methods would be useful to implement preventive measures at an early stage (15, 94, 149, 175).

4.3.5 Comparison of free-living planktonic and biofilm-associated bacteria

To get an impression of the ratio of free-living planktonic and biofilm-associated bacteria, the concentrations determined were related to the total basin area and to the total basin volume (for area and volume data see Table 2). For this purpose, the individual concentrations were multiplied by the corresponding and averaged for all cooling towers. Since no data were known for the entire piping network and the surfaces in the heat exchangers, the system volume was not related to the entire surface of the cooling system but only to the cooling tower basin. Thus, the values listed in Table 61 are approximations. No exact areas and volumes were determined and the volumes and areas examined will probably not represent the biofilm formation in the entire cooling system.

Table 61: Relation of biofilm-associated and planktonic microorganisms in [%].

[%]	Location	Mean CTs	CT1	CT2	CT3	CT4
HPC 22 °C	Biofilm	5.63	0.73	2.16	15.21	4.42
	planktonic	94.37	99.27	97.84	84.79	95.58
HPC 36 °C	Biofilm	19.59	1.77	2.44	67.65	6.48
	planktonic	80.41	98.23	97.56	32.35	93.52
***Legionella* spp.**	sessile	3.75	0.03	0.09	14.77	0.12
	planktonic	96.25	99.97	99.91	85.23	99.88
L. pneumophila	Biofilm	11.70	0.15	0.09	46.43	0.24
	planktonic	88.30	99.85	99.91	53.66	99.76
P. aeruginosa	Biofilm	47.03	58.42	92.84	80.63	17.48
	planktonic	52.97	41.42	7.16	19.73	82.52

Averaged over all cooling towers, the proportion of HPC at the incubation temperature of 22 °C was 5.63 % for sessile microorganisms located in the biofilm and 94.37 % for the planktonic HPC. In Cooling Tower 1, the fewest bacteria were biofilm-associated with 0.73 %, in Cooling Tower 3 with 15.21 % most bacteria were biofilm-associated. For HPC at 36 °C, an average of 19.59 % of the detected microorganisms is biofilm-associated and 80.41 % in the free water

phase. Also for HPC at 36 °C the fewest biofilm associated bacteria were found in Cooling Tower 1 (1.77 %) and the most (67.65 %) in Cooling Tower 3. For the comparison of biofilm-associated and planktonic *Legionella* spp. and *L. pneumophila* the water sample results calculated with the ISO method according to UBA colony counts were used. For *Legionella* spp. the proportion of surface-associated bacteria was 3.75 % and 96.25 % for planktonic ones. The proportion of biofilm-associated *L. pneumophila* amounted to 11.70 % and 88.30 % to free-living bacteria. In Cooling Tower 1 the fewest *Legionella* spp. (0.03 %) and in Cooling Tower 2 the fewest *L. pneumophila* (0.09 %) were sessile. In Cooling Tower 3, most surface-bound *Legionella* spp. (14.77 %) and *L. pneumophila* (46.34 %) were calculated. Also for the comparison of biofilm-associated *P. aeruginosa,* the ISO results of the water samples were used. Averaged over all cooling towers, the proportion of sessile bacteria was 47.03 % and of planktonic bacteria 52.97 %. In Cooling Tower 4 the fewest (17.48 %) *P. aeruginosa* were biofilm-associated, in Cooling Tower 2 (92.84 %) the most.

The discrepancy between biofilm-associated and planktonic *Legionella* spp. and *L. pneumophila* will probably be explained by the fact that the method used for the biofilm suspensions selected *L. pneumophila* over other *Legionella species* due to the combination of acid treatment and GVPC agar. The heat treatment applied to water samples promoted the growth of other *Legionella* species (see Figure 63).

In the final report on investigations in drinking water-associated biofilms was approximately determined that of the total biomass contained in the water system, 95 % is localized in the biofilm and 5 % in the water (43). This is not consistent with the determined proportions described above. In an artificial cooling tower with exactly known dimensions, the proportion of planktonic microorganisms was 20 % (116). The authors justified the comparatively higher value of planktonic organisms with the cooling water temperature range and lack of biocide treatment. These conditions were ideal for the microorganisms in the planktonic state (116). They also resumed that the high surface volume ratio that was given in their system is known to promote cell release from the biofilm into the water.

The biofilms grown on the plates located in high-turbulent flow areas have biased the results, as they underrepresent the total biofilm formation in the system. Biofilms preferably form in areas of low water flow and where water is allowed to stagnate, in areas with high nutrient availability and in warmer regions (3, 9, 146). Presumably, the ratio of planktonic to sessile microorganisms would be shifted in favour of the sessile ones, if the total surfaces of the sys-

tems (the pipe system, the heat exchangers and the fills) were known. Higher biocide concentrations are permitted in cooling systems than in drinking water systems. Therefore, using a capable biofilm removing biocide might cause planktonic concentrations higher than 5 %.

Initially it was planned to determine the biofilm mass on the last long-term plates after the ten-month observation period from all cooling towers. In cooling towers 1, 2 and 4 so little biomass was obtained that the tare value of the Petri dish weighed more than Petri dish plus biomass. Only the biomass in Cooling Tower 3 was determinable:

stainless steel plate: 10.224 g/100 cm^2 * 100 * 609 m^2 = 622.642 kg
polyethylene plate: 6.143 g/100 cm^2 * 100 * 609 m^2 = 374.109 kg

In average 498.4 kg biomass has formed on the basin surface area of Cooling Tower 3. Because culture methods are not able to detect all microorganisms in a sample due to their specific growth conditions, the mean heterotrophic plate counts of the observation period determined in water samples were used to compare the microbial mass in the cooling tower water with the biofilm mass. The mean number of HPC for both incubation temperatures in Cooling Tower 3 was generously rounded to 3,000 cfu/mL. Referred to the total basin volume, this amounts to 2.3 * 10^{12} cfu/760 m^3. A typical mass of a bacterium ranges about 10^{-12} g (43). Accordingly, in Cooling Tower 3 the mass of HPC was determined at about 2.3 g. The HPCs represent about 1 % of the total cell count (64, 157). Finally, the biomass of planktonic bacteria was estimated to 230 g in the entire cooling tower basin. Of the total biomass contained in the cooling tower basin, 99.95 % is located in biofilm and 0.05% in the free water. For drinking water networks a biomass ratio of 95 % to 5 % of biofilm to free water phase was estimated (43). Proportionally, there were 100 times more microorganisms located in the biofilm of cooling water systems than in the biofilm of drinking water systems.

Another approach to estimate the ratio of sessile biofilm-bound and planktonic microorganisms was made by relating the HPC of the long-term plates [cfu/25 cm^2] from Cooling Tower 3 to the total cooling tower basin area. The heterotrophic plate count per total cooling tower basin area was multiplied by the weight per cfu (10^{-12} g (43)). The percentage of the determined weight was related to the total mass of the biofilm (498.4 kg). The mean percentage of HPC in the total biofilm mass amounted to 0.0003 %. Assuming that the proportion of HPC in the free water is the same as in the biofilm, i.e. 2.3 g HPC form a proportion of 0.0003 % of the total mass of planktonic microorganisms, a total of 766.666 kg of microorganisms were present in the water of the cooling tower basin. This would mean that 1 m^3 of cooling water contained about 1 kg of microorganisms and a total of 1,265.1 kg microorganisms were present in Cooling Tower 3.The sessile, biofilm-bound microorganisms would have a proportion of 40 % and the planktonic microorganisms would have a proportion of 60 %. It seems extremely unrealistic

that 1 m^3 of cooling water contained 1 kg of microorganisms. A corresponding turbidity was not observed over the whole observation period, so that this second approach to calculate the biofilm-water ratio does not seem plausible. The calculation was probably influenced by too many artefacts during sampling, preparation of the suspension, and the uneven growth of the plates.

In summary, no realistic ratio of planktonic to sessile microorganisms was determined using these calculation models. It is assumed that caused by the biocide treatment the proportion of planktonic microorganisms is higher than 5 % as described for drinking water. Presumably, the bias was caused by the fact that the biofilm formation on the plates located in high turbulent flow areas did not represent the biofilm formation of the entire system.

5 Conclusions

Well-monitored cooling towers do not pose a high risk of causing legionellosis outbreaks. Since in well-monitored, biocide-treated cooling towers, long-term biofilm growth is controlled to the extent that it reflects the biofilm formed within four weeks. Only above a certain biofilm thickness, the biocide does not seem to be able to limit biofilm growth resulting in an increase of planktonic microorganism leaving the biofilm.

To improve the cooling tower surveillance and risk assessment retrospective correlation analyses of microbiological and process parameter data may offer an additional monitoring tool. Furthermore, an easy risk factor calculation based on previous sample testing of *Legionella* (and heterotrophic plate counts), which is highly modifiable by addition of other microbiological and process parameter data can show the cooling tower specific risk to cause legionellosis or the risk of not functioning properly depending on the choice of influencing parameters.

An improved cooling tower water monitoring is provided by the Idexx most probable number methods for the detection of *L. pneumophila* and *P. aeruginosa*. Both methods represented good alternatives to the recommended ISO procedures, as they are easy in handling and in result interpretation. Legiolert showed significantly higher results than the ISO method for *L. pneumophila* counts offering a better protection of the public health.

The laborious counting of heterotrophic microorganisms growing under ISO conditions and the production of time and material consuming dilution series is sufficient for one incubation temperature. The choice of incubation temperature of 22 or 36 °C should be based on the average cooling water temperature and/or validation tests depending on quantifiability.

Biofilm experiments and retrospective *Legionella* and HPC data indicated that both the microbial community and seasonal dynamics are cooling tower specific probably depending in particular on abiotic factors such as increased water or air temperature, nutrient input from make-up water or from the process, ambient air and, of course, the biocide conditions. Nevertheless, in all cooling towers *Legionella* tended to increase in late summer/autumn and HPC in mid-summer. The biofilm experiments on the polyethylene and stainless steel plates indicated, that the biocide has a higher impact on the biofilm formation and composition than the construction material. In cooling water systems the proportion of planktonic microorganisms is higher and the proportion of sessile microorganisms is lower than in drinking water systems. Presumably, more microorganisms are released from the thicker biofilms in cooling systems especially by higher biocide concentrations than in drinking water networks. Very low microbiological planktonic and sessile concentrations were detected in the cooling towers treated with chlorine dioxide and ozone.

6 Bibliography

1. 42. BImSchV - Zweiundvierzigste Verordnung zur Durchführung des Bundes-Immissionsschutzgesetzes (Verordnung über Verdunstungskühlanlagen, Kühltürme und Nassabscheider vom 12. Juli 2017 (BGBl. I S. 2379; 2018 I S. 202))
2. Ambrose J, Hampton LM, Fleming-Dutra KE, Marten C, McClusky C, et al. 2014. Large outbreak of Legionnaires' disease and Pontiac fever at a military base. Epidemiol. Infect. 142(11):2336–46
3. Apilánez I, Gutiérrez A, Díaz M. 1998. Effect of surface materials on initial biofilm development. Bioresour. Technol. 66(3):225–30
4. Armero C, Artacho A, López-Quílez A, Verdejo F. 2011. A probabilistic expert system for predicting the risk of Legionella in evaporative installations. Expert Syst. Appl. 38(6):6637–43
5. Bartram J, Bentham R, Briand E, Callan P, Crespi S, et al. 2007. Chapter 3 Approaches to risk management. In Bartram, J., Chartier, Y., Lee, J.V., Bond, K., Surman-Lee, S. : Legionella and the prevention of legionellosis, pp. 37–56. Geneva: World Health Organization
6. Beauté J, on behalf of the European Legionnaires' Disease Surveillance Network. 2017. Legionnaires' disease in Europe, 2011 to 2015. Eurosurveillance. 22(27):1–8
7. Bédard E, Prévost M, Déziel E. 2016. Pseudomonas aeruginosa in premise plumbing of large buildings. MicrobiologyOpen. 5(6):937–56
8. Bentham RH. 1993. Environmental factors affecting the colonization of cooling towers by Legionella spp. in South Australia. Int. Biodeterior. Biodegrad. 31(1):55–63
9. Bentham RH, Broadbent CR. 1993. A model for autumn outbreaks of Legionnaires' disease associated with cooling towers, linked to system operation and size. Epidemiol. Infect. 111(2):287–95
10. Berjeaud J-M, Chevalier S, Schlusselhuber M, Portier E, Loiseau C, et al. 2016. Legionella pneumophila: The Paradox of a Highly Sensitive Opportunistic Waterborne Pathogen Able to Persist in the Environment. Front. Microbiol. 7(486):
11. Bott TR. 1998. Techniques for reducing the amount of biocide necessary to counteract the effects of biofilm growth in cooling water systems. Appl. Therm. Eng. 18(11):1059–66
12. Castilla J, Barricarte A, Aldaz J, Garcíia Cenoz M, Ferrer T, et al. 2008. A large Legionnaires' disease outbreak in Pamplona, Spain: early detection, rapid control and no case fatality. Epidemiol. Infect. 136(6):823–32
13. Che D, Decludt B, Campese C, Desenclos JC. 2003. Sporadic cases of community acquired legionnaires' disease: an ecological study to identify new sources of contamination. J. Epidemiol. Community Health. 57(6):466–69
14. Chlordioxid macht die Kühlwasserdesinfektion sicherer und wirtschaftlicher. Aufgerufen am 18.08.2020. www.process.vogel.de
15. Costerton JW. 1999. Bacterial Biofilms: A Common Cause of Persistent Infections. Science. 284(5418):1318–22
16. Costerton JW, Lewandowski Z. 1997. The Biofilm Lifestyle. Adv. Dent. Res. 11(1):192–95
17. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. 1995. Microbial Biofilms. Annu. Rev. Microbiol. 49(1):711–45

18. Cunha BA, Burillo A, Bouza E. 2016. Legionnaires' disease. The Lancet. 387(10016):376–85

19. Cunha CB, Cunha BA. 2017. Legionnaire's Disease, An Issue of Infectious Disease Clinics of North America, E-Book. Elsevier Health Sciences. 225 pp.

20. Dang H, Lovell CR. 2016. Microbial Surface Colonization and Biofilm Development in Marine Environments. Microbiol. Mol. Biol. Rev. 80(1):91–138

21. Declerck P, Behets J, Margineanu A, van Hoef V, De Keersmaecker B, Ollevier F. 2009. Replication of Legionella pneumophila in biofilms of water distribution pipes. Microbiol. Res. 164(6):593–603

22. DGUV Information. 2014. Richtiger Umgang mit Dip-Slides - Wachstumsverlaufskontrolle von Mikroorganismen in wassergemischten KSS. DGUV Fachbereich Holz und Metall. FB HM-056

23. Di Gregorio L, Tandoi V, Congestri R, Rossetti S, Di Pippo F. 2017. Unravelling the core microbiome of biofilms in cooling tower systems. Biofouling. 33(10):793–806

24. Diederen BMW. 2008. Legionella spp. and Legionnaires' disease. J. Infect. 56(1):1–12

25. Domingue EL, Tyndall RL, Mayberry WR, Pancorbo OC. 1988. Effects of three oxidizing biocides on Legionella pneumophila serogroup 1. Appl. Environ. Microbiol. 54(3):741–47

26. Donlan R. 2002. Biofilms: Microbial Life on Surfaces. Emerg. Infect. Dis. 8:881–90

27. Duda S, Baron JL, Wagener MM, Vidic RD, Stout JE. 2015. Lack of correlation between Legionella colonization and microbial population quantification using heterotrophic plate count and adenosine triphosphate bioluminescence measurement. Environ. Monit. Assess. 187(7):393

28. Duda S, Stout JE, Vidic R. 2011. Biological control in cooling water systems using nonchemical treatment devices. HVAC&R Research. 17(5):872–90

29. DVGW-Technologizentrum Wasser. 2016. Bewertung der Risiken von Bioziden und ähnlich toxischen Einsatzstoffen aus dem Betrieb von offenen Kühltürmen für die Trinkwasserversorgung. AWBR Arbeitsgemeinschaft Wasserwerke Bodensee-Rhein, Karlsruhe

30. Eisenreich W, Heuner K. 2016. The life stage-specific pathometabolism of Legionella pneumophila. FEBS Lett. 590(21):3868–86

31. Engelhart S, Pleischl S, Lück C, Marklein G, Fischnaller E, et al. 2008. Hospital-acquired legionellosis originating from a cooling tower during a period of thermal inversion. Int. J. Hyg. Environ. Health. 211(3):235–40

32. European Center for Disease Prevention and Control (ECDC): Legionnaires' disease; annual epidemiological report for 2017. 2019

33. Exner M. 2020. Abteilungs-interne Lagebesprechung im Rahmen von Legionellosen in der Uniklinik Köln im Juli 2020.

34. Exner M, Kramer MH, Pleischl S. 2014. Strategies for Prevention and Control of Legionnaires' Disease in Germany. In Legionella, pp. 385–90. John Wiley & Sons, Ltd

35. Exner M, Pleischl S. 2010. Empfehlungen zur Gefährdungsbeurteilung und zur Prävention und Kontrolle von Legionellen in Rückkühlwerken. Umweltmed. Forsch. Prax. 15(4):193–201

36. Exner M, Suchenwirth R, Pleischl S, Kramer A, Eikmann T, et al. 2010. Memorandum zu dem Legionellen-Ausbruch in Ulm 2010 aus Sicht von Hygiene und Öffentlicher Gesundheit. Umweltmed Forsch Prax. 15(1):43–57

37. Faccini M, Russo AG, Bonini M, Tunesi S, Murtas R, et al. 2020. Large community-acquired Legionnaires' disease outbreak caused by Legionella pneumophila serogroup 1, Italy, July to August 2018. Eurosurveillance. 25(20):

38. Fernández J, López P, Orozco D, Merino J. 2002. Clinical Study of an Outbreak of Legionnaire's Disease in Alcoy, Southeastern Spain. Eur. J. Clin. Microbiol. Infect. Dis. 21(10):729–35

39. Feuerpfeil I, Botzenhart K. 2012. Hygienisch-mikrobiologische Wasseruntersuchung in der Praxis: Nachweismethoden, Bewertungskriterien, Qualitätssicherung, Normen. John Wiley & Sons. 208 pp.

40. Fields BS. The Social Life of Legionellae -. In R. Marre, Y. Abu Kwaik, C. Bartlett, N. P. Cianciotto, B. S. Fields, M. Frosch, J. Hacker, and P. C. Luck (ed.), Legionella., pp. 135–42. Washington, D.C.: ASM Press

41. Fields BS, Benson RF, Besser RE. 2002. Legionella and Legionnaires' Disease: 25 Years of Investigation. Clin. Microbiol. Rev. 15(3):506–26

42. Fields BS, Geary DF, McCoy WF, Bentham R, Lee JV. 2007. Chapter 5 Cooling towers and evaporative condensers. In Bartram, J., Chartier, Y., Lee, J.V., Bond, K., Surman-Lee, S.: Legionella and the prevention of legionellosis, pp. 69–88. Geneva: World Health Organization

43. Flemming H-C. 2003. Erfassung des Wachstums und des Kontaminationspotentials von Biofilmen in der Verteilung von Trinkwasser. Berichte aus dem Rheinisch-Westfälischen Institut für Wasserchemie und Wassertechnologie. Institut an der Gerhard-Mercator - Universität Duisburg, Mühlheim an der Ruhr

44. Flemming H-C, Murthy PS, Venkatesan R, Cooksey KE. 2008. Marine and Industrial Biofouling. Springer Science & Business Media. 330 pp.

45. Flemming H-C, Neu TR, Wozniak DJ. 2007. The EPS Matrix: The "House of Biofilm Cells." J. Bacteriol. 189(22):7945–47

46. Fraser DW, Tsai TR, Orenstein W, Parkin WE, Beecham HJ, et al. 1977. Legionnaires' Disease: Description of an Epidemic of Pneumonia. N. Engl. J. Med. 297(22):1189–97

47. Freudenmann M, Kurz S, von Baum H, Reick D, Schreff AM, et al. 2011. Interdisziplinäres Management eines länderübergreifenden Legionellenausbruchs. Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz. 54(11):1161

48. Galal-Gorchev H. 1996. Chlorine in Water Disinfection. Pure Appl. Chem. 68(9):1731–35

49. García-Fulgueiras A, Navarro C, Fenoll D, García J, González-Diego P, et al. 2003. Legionnaires' Disease Outbreak in Murcia, Spain. Emerg. Infect. Dis. 9(8):915–21

50. Gilmour MW, Bernard K, Tracz DM, Olson AB, Corbett CR, et al. 2007. Molecular typing of a Legionella pneumophila outbreak in Ontario, Canada. J. Med. Microbiol. 56(Pt 3):336–41

51. Glick TH, Gregg MB, Berman B, Mallison G, Rhodes WW, Kassanoff I. 1978. An Epidemic of unknown etiology in a health department: I. clinical and epidemiologic aspects. Am. J. Epidemiol. 107(2):149–60

52. Google Maps. Aufgerufen am 08.06.2020. www.google.de

53. Grant DM, Bott TR. 2005. Biocide Dosing Strategies for Biofilm Control. Heat Transf. Eng. 26(1):44–50

54. Greig JE, Carnie JA, Tallis GF, Zwolak B, Hart WG, et al. 2004. An outbreak of Legionnaires' disease at the Melbourne Aquarium, April 2000: investigation and case–control studies. Med. J. Aust. 180(11):566–72

55. Grohmann AN, Jekel M, Grohmann A, Szewzyk U, Szewzyk R. 2011. Wasser: Chemie, Mikrobiologie und nachhaltige Nutzung. Walter de Gruyter. 383 pp.

56. Grund G, Rahtz H, Korte H. 2017. Method of treating water with chlorine dioxide. United States Patent. US 9,743,670 B2

57. Hamilton KA, Haas CN. 2016. Critical review of mathematical approaches for quantitative microbial risk assessment (QMRA) of Legionella in engineered water systems: research gaps and a new framework. Environ. Sci. Water Res. Technol. 2(4):599–613

58. Hamilton KA, Prussin AJ, Ahmed W, Haas CN. 2018. Outbreaks of Legionnaires' Disease and Pontiac Fever 2006–2017. Curr. Environ. Health Rep. 5(2):263–71

59. Hammami N, Laisnez V, Wybo I, Uvijn D, Broucke C, et al. 2019. A cluster of Legionnaires' disease in Belgium linked to a cooling tower, August–September 2016: practical approach and challenges. Epidemiol. Infect. 147(e326):1–7

60. Hemmerich WA. Normalverteilung online prüfen | StatistikGuru. Zuletzt aufgerufen am 22.07.2020. https://statistikguru.de

61. Hemmerich WA. Korrelation online berechnen | StatistikGuru. Zuletzt aufgerufen am 22.07.2020. https://statistikguru.de

62. Hemmerich WA. Interquartilsabstand. Zuletzt aufgerufen am 22.07.2020. MatheGuru. https://matheguru.com

63. Hilbi H, Hoffmann C, Harrison CF. 2011. Legionella spp. outdoors: colonization, communication and persistence: Environmental communication of Legionella. Environ. Microbiol. Rep. 3(3):286–96

64. Hoefel D. 2003. Enumeration of water-borne bacteria using viability assays and flow cytometry: a comparison to culture-based techniques. J. Microbiol. Methods. 55(3):585–97

65. Homogeneity of Variance Calculator - Levene's Test. Zuletzt aufgerufen am 26.07.2020. www.socscistatistics.com

66. Hornei B, Ewig S, Exner M, Tartakovsky I, Lajoie L, et al. 2007. Chapter 1 Legionellosis. In Bartram, J., Chartier, Y., Lee, J.V., Bond, K., Surman-Lee, S.: Legionella and the prevention of legionellosis, pp. 1–28. Geneva: World Health Organization

67. Howland EB, Pope DH. 1983. Distribution and seasonality ofLegionella pneumophila in colling towers. Curr. Microbiol. 9(6):319–23

68. Hugosson A, Hjorth M, Bernander S, Claesson BEB, Johansson A, et al. 2007. A community outbreak of Legionnaires' disease from an industrial cooling tower: Assessment of clinical features and diagnostic procedures. Scand. J. Infect. Dis. 39(3):217–24

69. Iervolino M, Mancini B, Cristino S. 2017. Industrial Cooling Tower Disinfection Treatment to Prevent Legionella spp. Int. J. Environ. Res. Public. Health. 14(10):1125

70. Institut für Hygiene und Öffentliche Gesundheit. Current version. QM-Verfahrensanweisung: Probenahme allgemein.

71. Institut für Hygiene und Öffentliche Gesundheit. Current version. QM-Verfahrensanweisung: Probenahme aus Kühl- und Prozesswasserkeisläufen.

72. International Organization for Standardization. 1998. Water quality - Detection and enumeration of Legionella. ISO 11731:1998

73. International Organization for Standardization. 1999. Water quality - Enumeration of culturable microorganisms - Colony count by inoculation in a nutrient agar culture mediaum. ISO 6222:1999. German version EN ISO:1999

74. International Organization for Standardization. 2004. Water quality - Detection and enumeration of Legionella - Part 2: Direct membrane filtration method for waters with low bacterial counts. ISO 11731-2:2004

75. International Organization for Standardization. 2006. Water quality - Sampling for microbiological analysis. ISO 19458:2006. German version EN ISO:2006

76. International Organization for Standardization. 2007. Water quality – General guidance on the enumeration of micro-organisms by culture. ISO 8199: 2005. German version EN ISO:2007

77. International Organization for Standardization. 2008. Water quality - Detection and enumeration of Pseudomonas aeruginosa - Method by membrane filtration. ISO 16266:2006. German version EN ISO:2008

78. International Organization for Standardization. 2014. Water quality - Enumeration of Escherichia coli and coliform bacteria - Part 2: Most probable number method (ISO 9308-2:2012); German version EN ISO 9308-2:2014

79. International Organization for Standardization. 2014. Water quality – Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods. ISO 17994:2014

80. International Organization for Standardization. 2017. Water quality - Enumeration of Legionella. ISO 11731:2017

81. International Organization for Standardization. 2018. Water quality - General requirements and guidance for microbiological examinations by culture. ISO 8199:2018

82. International Organization for Standardization. 2018. Water quality – Detection and enumeration of Pseudomonas aeruginosa – Part 2: Most probable number method. ISO 16266-2:2018

83. Jansà J. M, Caylà J. A, Ferrer D, Gracia J, Pelaz C, et al. 2002. An outbreak of Legionnaires' disease in an inner city district: importance of the first 24 hours in the investigation. Int. J. Tuberc. Lung Dis. 6(9):831–38

84. Keller D. 2013. Analyse von Zusammenhängen: Korrelation. Abgerufen am 07.07.2020. https://statistik-und-beratung.de/2013/05/analyse-von-zusammenhangen-korrelation/

85. Keramarou M, Evans MR, for the South Wales Legionnaires' D C. 2010. A community outbreak of Legionnaires' disease in South Wales, August–September 2010. Eurosurveillance. 15(42):19691

86. Kerr CJ, Osborn KS, Robson GD, Handley PS. 1998. The relationship between pipe material and biofilm formation in a laboratory model system. J. Appl. Microbiol. 85(S1):29S-38S

87. Kirrage D, Reynolds G, Smith GE, Olowokure B. 2007. Investigation of an outbreak of Legionnaires' disease: Hereford, UK 2003. Respir. Med. 101(8):1639–44

88. Kirschner AKT, Rameder A, Schrammel B, Indra A, Farnleitner AH, Sommer R. 2012. Development of a new CARD-FISH protocol for quantification of Legionella pneumophila and its application in two hospital cooling towers. J. Appl. Microbiol. 112(6):1244–56

89. Ko KS, Lee HK, Park M-Y, Lee K-H, Yun Y-J, et al. 2002. Application of RNA Polymerase -Subunit Gene (rpoB) Sequences for the Molecular Differentiation of Legionella Species. J. Clin. Microbiol. 40(7):2653–58

90. Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein Westfalen. 2020. Ringversuch "Legionellen und allgemeine Koloniezahlen in Kühlwasser." Recklinghausen

91. Legionnaires Disease and Pontiac Fever | Legionella | CDC. Zuletzt aufgerufen am 22.05.2020. www.cdc.gov

92. Lewis K. 2001. Riddle of Biofilm Resistance. Antimicrob. Agents Chemother. 45(4):999–1007

93. Liu L, Xu Y, Cui F, Xia Y, Chen L, et al. 2018. Monitoring of bacteria biofilms forming process by in-situ impedimetric biosensor chip. Biosens. Bioelectron. 112:86–92

94. Liu Y, Zhang W, Sileika T, Warta R, Cianciotto NP, Packman A. 2009. Role of bacterial adhesion in the microbial ecology of biofilms in cooling tower systems. Biofouling. 25(3):241–53

95. Llewellyn AC, Lucas CE, Roberts SE, Brown EW, Nayak BS, et al. 2017. Distribution of Legionella and bacterial community composition among regionally diverse US cooling towers. PLOS ONE. 12(12):e0189937

96. Lopez D, Vlamakis H, Kolter R. 2010. Biofilms. Cold Spring Harb. Perspect. Biol. 2(7):a000398

97. Maisa A, Brockmann A, Renken F, Lück C, Pleischl S, et al. 2015. Epidemiological investigation and case–control study: a Legionnaires' disease outbreak associated with cooling towers in Warstein, Germany, August–September 2013. Eurosurveillance. 20(46):30064

98. Mampel J, Spirig T, Weber SS, Haagensen JAJ, Molin S, Hilbi H. 2006. Planktonic Replication Is Essential for Biofilm Formation by Legionella pneumophila in a Complex Medium under Static and Dynamic Flow Conditions. Appl. Environ. Microbiol. 72(4):2885–95

99. Mann-Whitney U Test Calculator. Zuletzt aufgerufen am 26.07.2020. www.socscistatistics.com

100. McCarthy M. 2001. Breaking up the bacterial happy home. The Lancet. 357(9273):2032

101. McCormick D, Thorn S, Milne D, Evans C, Stevenson J, et al. 2012. Public health response to an outbreak of Legionnaires' disease in Edinburgh, United Kingdom, June 2012. Eurosurveillance. 17(28):20216

102. McCoy WF. 2005. Preventing Legionellosis. IWA Publishing. 151 pp.

103. McDade JE, Shepard CC, Fraser DW, Tsai TR, Redus MA, Dowdle WR. 1977. Legionnaires' Disease. N. Engl. J. Med. 297(22):1197–1203

104. McLeod SA. What does effect size tell you? | Simply Psychology. Zuletzt aufgerufen am 08.07.2020. What does effect size tell you? www.simplypsychology.org

105. McNemar Q. 1947. Note on the sampling error of the difference between correlated proportions or percentages. Psychometrika. 12(2):153–57

106. Miller MB, Bassler BL. 2001. Quorum Sensing in Bacteria. Annu. Rev. Microbiol. 55(1):165–99

107. Miller RD, Kenepp KA. 1993. Risk Assessments for Legionnaires' Disease based on routine surveillance of cooling towers for Legionellae. In Barbaree, J.M., Breimann, R.F., Dufour, R.F. (eds.), Legionella Current Status and Emerging Perspectives, pp. 40–43. USA: ASM

108. Momba MN, Makala N. 2004. Comparing the effect of various pipe materials on biofilm formation in chlorinated and combined chlorine-chloraminated water systems. Water SA. 30(2):175–82

109. Mouchtouri V, Goutziana G, Kremastinou J, Hadjichristodoulou C. 2009. Legionella species colonization in cooling towers: Risk factors and assessment of control measures. Am. J. Infect. Control. 38:50–55

110. Murga R, Forster TS, Brown E, Pruckler JM, Fields BS, Donlan RM. 2001. Role of biofilms in the survival of Legionella pneumophila in a model potable-water system. Microbiology. 147(11):3121–26

111. Musk Jr. D, Hergenrother P. 2006. Chemical Countermeasures for the Control of Bacterial Biofilms: Effective Compounds and Promising Targets. Curr. Med. Chem. 13(18):2163–77

112. Nhu Nguyen TM, Ilef D, Jarraud S, Rouil L, Campese C, et al. 2006. A Community-Wide Outbreak of Legionnaires Disease Linked to Industrial Cooling Towers—How Far Can Contaminated Aerosols Spread? J. Infect. Dis. 193(1):102–11

113. Nygård K, Werner-Johansen Ø, Rønsen S, Caugant DA, Simonsen Ø, et al. 2008. An Outbreak of Legionnaires Disease Caused by Long-Distance Spread from an Industrial Air Scrubber in Sarpsborg, Norway. Clin. Infect. Dis. 46(1):61–69

114. O'Brien S, Bhopal R. 1993. Legionnaires' disease: the infective dose paradox. The Lancet. 342(8862):5–6

115. Oder M, Fink R, Bohinc K, Torkar KG. 2017. The influence of shear stress on the adhesion capacity of Legionella pneumophila. Arch. Ind. Hyg. Toxicol. 68(2):109–15

116. Paniagua AT, Paranjape K, Hu M, Bédard E, Faucher SP. 2020. Impact of temperature on Legionella pneumophila, its protozoan host cells, and the microbial diversity of the biofilm community of a pilot cooling tower. Sci. Total Environ. 712:136131

117. Paranjape K, Bédard É, Whyte LG, Ronholm J, Prévost M, Faucher SP. 2020. Presence of Legionella spp. in cooling towers: the role of microbial diversity, Pseudomonas, and continuous chlorine application. Water Res. 169:115252

118. Pedersen K. 1990. Biofilm development on stainless steel and pvc surfaces in drinking water. Water Res. 24(2):239–43

119. Pereira RPA, Peplies J, Höfle MG, Brettar I. 2017. Bacterial community dynamics in a cooling tower with emphasis on pathogenic bacteria and Legionella species using universal and genus-specific deep sequencing. Water Res. 122:363–76

120. Phares CR, Russell E, Thigpen MC, Service W, Crist MB, et al. 2007. Legionnaires' disease among residents of a long-term care facility: The sentinel event in a community outbreak. Am. J. Infect. Control. 35(5):319–23

121. Pinel ISM, Kim LH, Borges VRP, Farhat NM, Witkamp G-J, et al. 2020. Effect of phosphate availability on biofilm formation in cooling towers. Biofouling. 0(0):1–16

122. Pires L, Sachsenheimer K, Kleintschek T, Waldbaur A, Schwartz T, Rapp BE. 2013. Online monitoring of biofilm growth and activity using a combined multi-channel impedimetric and amperometric sensor. Biosens. Bioelectron. 47:157–63

123. Prussin AJ, Schwake DO, Marr LC. 2017. Ten questions concerning the aerosolization and transmission of Legionella in the built environment. Build. Environ. 123:684–95

124. Quinn C, Demirjian A, Watkins LF, Tomczyk S, Lucas C, et al. 2015. Legionnaires' Disease Outbreak at a Long-Term Care Facility Caused by a Cooling Tower Using an Automated Disinfection System—Ohio, 2013. J. Environ. Health. 78(5):8–13

125. Ragull S, Montenegro R, García-Núñez M, Sanchez I, Soler A, et al. 2006. Fluctuation in Legionella pneumophila Counts in Cooling Towers over a 1-Year Period. In Legionella, pp. 436–38. John Wiley & Sons, Ltd

126. Rasamiravaka T, Labtani Q, Duez P, El Jaziri M. 2015. The Formation of Biofilms by Pseudomonas aeruginosa: A Review of the Natural and Synthetic Compounds Interfering with Control Mechanisms. BioMed Res. Int. 2015:e759348

127. Rech MM, Swalla BM, Dobranic JK. 2018. Evaluation of Legiolert for Quantification of Legionella pneumophila from Non-potable Water. Curr. Microbiol. 75(10):1282–89

128. Rickard AH, Gilbert P, High NJ, Kolenbrander PE, Handley PS. 2003. Bacterial coaggregation: an integral process in the development of multi-species biofilms. Trends Microbiol. 11(2):94–100

129. Ricketts KD, Joseph C, Lee J, Wewalka G, Infections EWG for L. 2008. Survey on legislation regarding wet cooling systems in European countries. Eurosurveillance. 13(38):18982

130. RKI - RKI-Ratgeber - Legionellose. Zuletzt aufgerufen am 31.08.2020. www.rki.de

131. Robert-Koch-Institut: Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2018. Berlin: 2019

132. Rogers J, Dowsett AB, Dennis PJ, Lee JV, Keevil CW. 1994. Influence of Plumbing Materials on Biofilm Formation and Growth of Legionella pneumophila in Potable Water Systems. Appl. Environ. Microbiol. 60(6):1842–51

133. Rota MC, Pontrelli G, Scaturro M, Bella A, Bellomo AR, et al. 2005. Legionnaires' disease outbreak in Rome, Italy. Epidemiol. Infect. 133(5):853–59

134. Sabria M, Alvarez J, Dominguez A, Pedrol A, Sauca G, et al. 2006. A community outbreak of Legionnaires' disease: evidence of a cooling tower as the source. Clin. Microbiol. Infect. 12(7):642–47

135. Sala Ferré MR, Arias C, Oliva JM, Pedrol A, García M, et al. 2008. A community outbreak of Legionnaires' disease associated with a cooling tower in Vic and Gurb, Catalonia (Spain) in 2005. Eur. J. Clin. Microbiol. Infect. Dis. 28(2):153

136. Sartory DP, Spies K, Lange B, Schneider S, Langer B. 2017. Evaluation of a most probable number method for the enumeration of Legionella pneumophila from potable and related water samples. Lett. Appl. Microbiol. 64(4):271–75

137. Schulte S, Wingender J, Flemming H-C. 2005. Efficacy of biocides against biofilms. In Directory of Microbicides for the Protection of Materials: A Handbook, ed W Paulus, pp. 93–120. Dordrecht: Springer Netherlands

138. Shivaji T, Sousa Pinto C, San-Bento A, Oliveira Serra LA, Valente J, et al. 2014. A large community outbreak of Legionnaires' disease in Vila Franca de Xira, Portugal, October to November 2014. Eurosurveillance. 19(50):20991

139. Simpson GD, Miller RF, Laxton GD, Clements WR. 1993. A focus on chlorine dioxide: the "ideal" biocide. Corrosion. 93:8-12. paper no 472

140. Søndergaard M. 2009. Redox Potential. In Encyclopedia of Inland Waters, ed GE Likens, pp. 852–59. Oxford: Academic Press

141. Spies K, Pleischl S, Lange B, Langer B, Hübner I, et al. 2018. Comparison of the Legiolert™/Quanti-Tray® MPN test for the enumeration of Legionella pneumophila from potable water samples with the German regulatory requirements methods ISO 11731-2 and ISO 11731. Int. J. Hyg. Environ. Health. 221(7):1047–53

142. Spies K, Pleischl S, Sartory D. 2020. Comparison of the Pseudalert™/Quanti-Tray® MPN test for the enumeration of Pseudomonas aeruginosa in cooling tower water with the ISO 16266 membrane filtration culture-based method. J. Appl. Microbiol. 128(6):1843–50

143. Stewart CR, Muthye V, Cianciotto N. 2012. Legionella pneumophila Persists within Biofilms Formed by Klebsiella pneumoniae, Flavobacterium sp., and Pseudomonas fluorescens under Dynamic Flow Conditions. PloS One. 7(11):e50560

144. Stoodley P, Sauer K, Davies DG, Costerton JW. 2002. Biofilms as Complex Differentiated Communities. Annu. Rev. Microbiol. 56(1):187–209

145. Stout JE, Yu VL. 1997. Legionellosis. N. Engl. J. Med. 337(10):682–87

146. Surman-Lee S, Fields BS, Hornei B, Ewig S, Exner M, et al. 2007. Chapter 2 Ecology and environmental sources of Legionella. In Bartram, J., Chartier, Y., Lee, J.V., Bond, K., Surman-Lee, S.: Legionella and the prevention of legionellosis, pp. 29–38. Geneva: World Health Organization

147. Taylor M, Ross K, Bentham R. 2013. Spatial Arrangement of Legionella Colonies in Intact Biofilms from a Model Cooling Water System. Microbiol. Insights. 6:MBI.S12196

148. Trinkwasserverordnung - German drinking water ordinance. Trinkwasserverordnung in der Fassung der Bekanntmachung vom 28. November 2011 (BGBI. I S. 2370), die durch Artikel 2 Absatz 19 des Gesetztes vom 22. Dezember 2011 (BGBI I S. 3044) geändert worden ist

149. Tsao H-F, Scheikl U, Herbold C, Indra A, Walochnik J, Horn M. 2019. The cooling tower water microbiota: Seasonal dynamics and co-occurrence of bacterial and protist phylotypes. Water Res. 159:464–79

150. T-Test Calculator for 2 Dependent Means. Zuletzt aufgerufen am 26.07.2020. www.socscistatistics.com

151. T-Test Calculator for 2 Independent Means. Zuletzt aufgerufen am 26.07.2020. www.socscistatistics.com

152. Türetgen I, Sungur EI, Cotuk A. 2005. Enumeration of Legionella pneumophila in cooling tower water systems. Environ. Monit. Assess. 100(1–3):53–58

153. Two Sample T-Test (Welch's T-test). Zuletzt aufgerufen am 26.07.2020. www.statskingdom.com

154. Umweltbundeamt. 2012. Empfehlung des Umweltbundesamtes nach Anhörung der Trinkwasserkommission: Systemische Untersuchungen von Trinkwasser-Installationen auf Legionellen nach Trinkwasserverordnung

155. Umweltbundesamt. 2017. Empfehlung des Umweltbundesamtes zur Probenahme und zum Nachweis von Legionellen in Verdunstungskühlanlagen, Kühltürmen und Nassabscheidern

156. Umweltbundesamt. 2020. Empfehlung des Umweltbundeamtess zur Probenahme und zum Nachweis von Legionellen in Verdunstungslühlanlagen, Kühltürmen und Nassabscheidern

157. Van Nevel S, Koetzsch S, Proctor CR, Besmer MD, Prest EI, et al. 2017. Flow cytometric bacterial cell counts challenge conventional heterotrophic plate counts for routine microbiological drinking water monitoring. Water Res. 113:191–206

158. Vatansever C, Türetgen I. 2018. Investigating the effects of different physical and chemical stress factors on microbial biofilm. Water SA. 44:308–17

159. Verein Deutscher Ingenieure. 2015. Rückkühlwerke - Sicherstellung des hygienegerechten Betriebs von Verdunstungslühlanlagen (VDI-Kühlturmregeln) - Open recooler systems Securing hygienically sound operation of evaporative cooling systems (VDI Cooling Tower Code of Practice)

160. Videla HA, Characklis WG. 1992. Biofouling and microbially influenced corrosion. Int. Biodeterior. Biodegrad. 29(3):195–212

161. Viera MR, Guiamet PS, de Mele MFL, Videla HA. 1999. Use of dissolved ozone for controlling planktonic and sessile bacteria in industrial cooling systems. Int. Biodeterior. Biodegrad. 44:201–7

162. Vital-Lopez FG, Reifman J, Wallqvist A. 2015. Biofilm Formation Mechanisms of Pseudomonas aeruginosa Predicted via Genome-Scale Kinetic Models of Bacterial Metabolism. PLOS Comput. Biol. 11(10):e1004452

163. von Baum H, Ewig S, Marre R, Suttorp N, Gonschior S, et al. 2008. Community-Acquired Legionella Pneumonia: New Insights from the German Competence Network for Community Acquired Pneumonia. Clin. Infect. Dis. 46(9):1356–64

164. Walser SM, Gerstner DG, Brenner B, Höller C, Liebl B, Herr CEW. 2014. Assessing the environmental health relevance of cooling towers – A systematic review of legionellosis outbreaks. Int. J. Hyg. Environ. Health. 217(2–3):145–54

165. Wang J, Liu M, Xiao H, Wu W, Xie M, et al. 2013. Bacterial community structure in cooling water and biofilm in an industrial recirculating cooling water system. Water Sci. Technol. 68(4):940–47

166. Watnick P, Kolter R. 2000. Biofilm, City of Microbes. J. Bacteriol. 182(10):2675–79

167. Weiss D, Boyd C, Rakeman JL, Greene SK, Fitzhenry R, et al. 2017. A Large Community Outbreak of Legionnaires' Disease Associated With a Cooling Tower in New York City, 2015. Public Health Rep. 132(2):241–50

168. Wewalka G, Schmid D, Harrison TG, Uldum SA, Lück C. 2014. Dual infections with different Legionella strains. Clin. Microbiol. Infect. 20(1):O13–19

169. White PS, Graham FF, Harte DJG, Baker MG, Ambrose CD, Humphrey ARG. 2013. Epidemiological investigation of a Legionnaires' disease outbreak in Christchurch, New Zealand: the value of spatial methods for practical public health. Epidemiol. Infect. 141(4):789–99

170. Wilcoxon Signed-Rank Test Calculator. Zuletzt aufgerufen am 26.07.2020. www.socscistatistics.com

171. Winter J, Ilbert M, Graf PCF, Özcelik D, Jakob U. 2008. Bleach Activates a Redox-Regulated Chaperone by Oxidative Protein Unfolding. Cell. 135(4):691–701

172. World Health Organization, ed. 2003. Heterotrophic plate counts and drinking-water safety: the significance of HPCs for water quality and human health. Edited by Bartram, J., Cotruvo, J., Exner, M., Frickler, C., Glasmacher, A. London, UK: IWA Publ. 256 pp.

173. Yamamoto H, Ezaki T, Ikedo M, Yabuuchi E. 1991. Effects of Biocidal Treatments to Inhibit the Growth of Legionellae and Other Microorganisms in Cooling Towers. Microbiol. Immunol. 35(9):795–802

174. Zacheus OM, Iivanainen EK, Nissinen TK, Lehtola MJ, Martikainen PJ. 2000. Bacterial biofilm formation on polyvinyl chloride, polyethylene and stainless steel exposed to ozonated water. Water Res. 34(1):63–70

175. Zeybek Z, Gungor ND, Turetgen İ. 2017. Investigation of Heterotrophic Bacteria, Legionella and Free-Living Amoeba in Cooling Tower Samples by FISH and Culture Methods. Eur. J. Biol. 76(1):7–13

7 Data Annex

Annex A: Data for the Legiolert-ISO-methods comparison (section 3.1)
Annex B: Data for the retrospective analyses (section 3.2)

Data Annex Contents

Annex A: Data for the Legiolert-ISO-methods comparison (section 3.1)

DA Table A 1: Sample information for Legiolert-ISO-methods comparison. 204
DA Table A 2:ISO method - *Legionella sp*. 206
DA Table A 3: ISO method - *L. pneumophila* counts. 208
DA Table A 4: Legiolert. 210

Annex B: Data for the retrospective analyses (section 3.2)

DA Table B 1: Cooling Tower 1 – Microbiological concentrations from the Laboratory of Technical Hygiene, IHPH. 213
DA Table B 2: Cooling Tower 1 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 5 days. 216
DA Table B 3: Cooling Tower 1 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 5 days. 218
DA Table B 4: Cooling Tower 1 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 7 days. 219
DA Table B 5: Cooling Tower 1 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 7 days. 221
DA Table B 6: Cooling Tower 1 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 10 days. 222
DA Table B 7: Cooling Tower 1 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 10 days. 224
DA Table B 8: Cooling Tower 1 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 5 days 225
DA Table B 9: Cooling Tower 1 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 5 days 227
DA Table B 10: Cooling Tower 1 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 7 days. 228
DA Table B 11: Cooling Tower 1 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 7 days. 230
DA Table B 12: Cooling Tower 1 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 10 days. 231
DA Table B 13: Cooling Tower 1 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 10 days. 233
DA Table B 14: Cooling Tower 2 – Microbiological concentrations from the Laboratory of Technical Hygiene, IHPH 234
DA Table B 15: Cooling Tower 2 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 350 mV, observation period: 5 days. 237
DA Table B 16: Cooling Tower 2 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 350 mV, observation period: 5 days. 239

DA Table B 17: Cooling Tower 2 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 350 mV, observation period: 7 days. 240

DA Table B 18: Cooling Tower 2 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 350 mV, observation period: 7 days. 242

DA Table B 19: Cooling Tower 2 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 350 mV, observation period: 10 days. 243

DA Table B 20: Cooling Tower 2 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 350 mV, observation period: 10 days. 245

DA Table B 21: Cooling Tower 2 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 5 days. 246

DA Table B 22: Cooling Tower 2 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 5 days. 248

DA Table B 23: Cooling Tower 2 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 7 days. 249

DA Table B 24: Cooling Tower 2 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 7 days. 251

DA Table B 25: Cooling Tower 2 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 10 days. 252

DA Table B 26: Cooling Tower 2 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 10 days. 254

DA Table B 27: Cooling Tower 3 –Microbiological concentrations from the Laboratory of Technical Hygiene, IHPH. 255

DA Table B 28: Cooling Tower 3 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 5 days. 258

DA Table B 29: Cooling Tower 3 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 5 days. 260

DA Table B 30: Cooling Tower 3 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 7 days. 261

DA Table B 31: Cooling Tower 3 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 7 days. 263

DA Table B 32: Cooling Tower 3 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 10 days. 264

DA Table B 33: Cooling Tower 3 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 10 days. 266

DA Table B 34: Cooling Tower 3 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 5 days. 267

DA Table B 35: Cooling Tower 3 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 5 days. 269

DA Table B 36: Cooling Tower 3 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 7 days. 270

DA Table B 37: Cooling Tower 3 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 7 days. 272

DA Table B 38: Cooling Tower 3 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 10 days. 273

DA Table B 39: Cooling Tower 3 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 10 days.....275

DA Table B 40: Cooling Tower 4 – Microbiological concentrations from the Laboratory of Technical Hygiene, IHPH. 276

DA Table B 41: Cooling Tower 4 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 5 days.....279

DA Table B 42: Cooling Tower 4 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 5 days. 281

DA Table B 43: Cooling Tower 4 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 7 days.....282

DA Table B 44: Cooling Tower 4 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 7 days. 284

DA Table B 45: Cooling Tower 4 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 10 days.....285

DA Table B 46: Cooling Tower 4 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 10 days. 287

DA Table B 47: Cooling Tower 4 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 25 °C, observation period: 5 days.....288

DA Table B 48: Cooling Tower 4 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 25 °C, observation period: 5 days.....290

DA Table B 49: Cooling Tower 4 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 25 °C, observation period: 7 days.....291

DA Table B 50: Cooling Tower 4 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 25 °C, observation period: 7 days.....293

DA Table B 51: Cooling Tower 4 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 25 °C, observation period: 10 days.....294

DA Table B 52: Cooling Tower 4 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 25 °C, observation period: 10 days.....296

DA Table B 53: Retrospective HPC data for the comparison of HPC at the incubation temperatures of 22 and 36 °C. 297

Data for the Legiolert-ISO-methods comparison (section 3.1)

DA Table A 1: Sample information for Legiolert-ISO-methods comparison.

Sample Number	Lab ID H2018-	Origin of Water Sample	Sampling Date	Processing Date (Legiolert and ISO)	Repeats of sampling site
1	5084	Cooling Tower	04.09.2018	06.09.2018	3
2	5091	Cooling Tower	04.09.2018	06.09.2018	4
3	5092	Cooling Tower	04.09.2018	06.09.2018	4
4	5093	Cooling Tower	04.09.2018	06.09.2018	3
5	5098	Cooling Tower	04.09.2018	06.09.2018	3
6	5099	Cooling Tower	04.09.2018	06.09.2018	3
7	5108	Process Water	05.09.2018	06.09.2018	3
8	5111	Cooling Tower	05.09.2018	06.09.2018	6
9	5112	Process Water	05.09.2018	06.09.2018	5
10	5113	Process Water	05.09.2018	06.09.2018	3
11	5114	Process Water	05.09.2018	06.09.2018	4
12	5115	Process Water	05.09.2018	06.09.2018	3
13	5246	Cooling Tower	11.09.2018	12.09.2018	3
14	5247	Cooling Tower	11.09.2018	12.09.2018	3
15	5248	Cooling Tower	11.09.2018	12.09.2018	3
16	5249	Cooling Tower	11.09.2018	12.09.2018	3
17	5250	Cooling Tower	11.09.2018	12.09.2018	2
18	5251	Cooling Tower	11.09.2018	12.09.2018	2
19	5252	Cooling Tower	11.09.2018	12.09.2018	2
20	5253	Cooling Tower	11.09.2018	12.09.2018	2
21	5254	Cooling Tower	11.09.2018	12.09.2018	2
22	5255	Cooling Tower	12.09.2018	12.09.2018	6
23	5256	Process Water	12.09.2018	12.09.2018	5
24	5258	Process Water	12.09.2018	12.09.2018	4
25	5424	Cooling Tower	17.09.2018	19.09.2018	2
26	5425	Cooling Tower	17.09.2018	19.09.2018	2
27	5431	Cooling Tower	17.09.2018	19.09.2018	2
28	5470	Process Water	19.09.2018	19.09.2018	2
29	5471	Cooling Tower	19.09.2018	19.09.2018	4
30	5473	Process Water	19.09.2018	19.09.2018	3
31	5475	Process Water	19.09.2018	19.09.2018	2
32	5476	Cooling Tower	19.09.2018	19.09.2018	6
33	5477	Process Water	19.09.2018	19.09.2018	5
34	5478	Process Water	19.09.2018	19.09.2018	3
35	5479	Process Water	19.09.2018	19.09.2018	4
36	5480	Process Water	19.09.2018	19.09.2018	3
37	5612	Cooling Tower	25.09.2018	26.09.2018	2
38	5613	Cooling Tower	25.09.2018	26.09.2018	2
39	5614	Cooling Tower	25.09.2018	26.09.2018	2
40	5615	Cooling Tower	25.09.2018	26.09.2018	2
41	5616	Cooling Tower	25.09.2018	26.09.2018	2
42	5617	Cooling Tower	25.09.2018	26.09.2018	2
43	5618	Cooling Tower	25.09.2018	26.09.2018	2
44	5709	Cooling Tower	26.09.2018	26.09.2018	2
45	5694	Cooling Tower	25.09.2018	26.09.2018	3
46	5695	Cooling Tower	25.09.2018	26.09.2018	3
47	5696	Cooling Tower	25.09.2018	26.09.2018	6
48	5702	Cooling Tower	25.09.2018	26.09.2018	4
49	5786	Cooling Tower	01.10.2018	02.10.2018	4

Sample Number	Lab ID H2018-	Origin of Water Sample	Sampling Date	Processing Date (Legiolert and ISO)	Repeats of sampling site
50	5813	Cooling Tower	01.10.2018	02.10.2018	3
51	5814	Cooling Tower	01.10.2018	02.10.2018	3
52	5815	Cooling Tower	01.10.2018	02.10.2018	3
53	5817	Cooling Tower	01.10.2018	02.10.2018	3
54	5818	Cooling Tower	01.10.2018	02.10.2018	3
55	5819	Cooling Tower	01.10.2018	02.10.2018	3
56	5820	Cooling Tower	01.10.2018	02.10.2018	3
57	5821	Cooling Tower	01.10.2018	02.10.2018	3
58	5822	Cooling Tower	01.10.2018	02.10.2018	3
59	5823	Cooling Tower	01.10.2018	02.10.2018	4
60	5824	Cooling Tower	01.10.2018	02.10.2018	4
61	5946	Cooling Tower	09.10.2018	11.10.2018	4
62	5947	Cooling Tower	09.10.2018	11.10.2018	3
63	5948	Cooling Tower	09.10.2018	11.10.2018	2
64	5949	Cooling Tower	09.10.2018	11.10.2018	2
65	5953	Cooling Tower	09.10.2018	11.10.2018	2
66	5955	Cooling Tower	09.10.2018	11.10.2018	2
67	5968	Cooling Tower	09.10.2018	11.10.2018	1
68	5969	Cooling Tower	09.10.2018	11.10.2018	2
69	5986	Cooling Tower	09.10.2018	11.10.2018	3
70	5987	Cooling Tower	09.10.2018	11.10.2018	3
71	5988	Cooling Tower	09.10.2018	11.10.2018	3
72	5989	Cooling Tower	09.10.2018	11.10.2018	3
73	6134	Cooling Tower	16.10.2018	17.10.2018	1
74	6135	Cooling Tower	16.10.2018	17.10.2018	1
75	6136	Cooling Tower	16.10.2018	17.10.2018	1
76	6137	Cooling Tower	16.10.2018	17.10.2018	1
77	6138	Cooling Tower	16.10.2018	17.10.2018	1
78	6169	Cooling Tower	17.10.2018	23.10.2018	1
79	6170	Cooling Tower	17.10.2018	23.10.2018	2
80	6180	Cooling Tower	18.10.2018	23.10.2018	1
81	6181	Cooling Tower	18.10.2018	23.10.2018	2
82	6223	Cooling Tower	22.10.2018	23.10.2018	1
83	6363	Cooling Tower	24.10.2018	01.11.2018	2
84	6365	Cooling Tower	24.10.2018	01.11.2018	2
85	6366	Cooling Tower	24.10.2018	01.11.2018	2
86	6403	Cooling Tower	25.10.2018	01.11.2018	1
87	6425	Cooling Tower	29.10.2018	01.11.2018	4
88	6426	Cooling Tower	29.10.2018	01.11.2018	3
89	6448	Cooling Tower	30.10.2018	01.11.2018	1
90	6449	Cooling Tower	30.10.2018	01.11.2018	1
91	6462	Cooling Tower	30.10.2018	01.11.2018	1
92	6477	Cooling Tower	30.10.2018	01.11.2018	3
93	6478	Cooling Tower	30.10.2018	01.11.2018	3
94	6493	Cooling Tower	31.10.2018	01.11.2018	4
95	6798	Wet Separator	13.11.2018	15.11.2018	2
96	6799	Wet Separator	13.11.2018	15.11.2018	3
97	6800	Wet Separator	13.11.2018	15.11.2018	3
98	6802	Wet Separator	13.11.2018	15.11.2018	2
99	6803	Wet Separator	13.11.2018	15.11.2018	3

DA Table A 2:ISO method - *Legionella sp*.

Sample Number	Lab ID H2018-	Final result [cfu/100 mL]	Number of cfu for Final Result Calculation	Treatment and Volume for Final Result Calculation	Serology (Latex Test Kit)
1	5084	2500	25	heat 1 mL	Lp
2	5091	<DL	0		
3	5092	2000	2	heat 0.1 mL	Lp
4	5093	1000	1	heat 0.1 mL	Lp
5	5098	4000	4	acid 0.1 mL	Lp
6	5099	25	5	acid MF 20 mL	Lp
7	5108	310	61	acid MF 20 mL	L. sp.
8	5111	15000	15	acid 0.1 mL	Lp +L.sp.
9	5112	2600	27	heat combined DP	Lp +L.sp.
10	5113	24000	239	heat combined DP	Lp
11	5114	5000	50	heat 1 mL	Lp +L.sp.
12	5115	1800	18	heat 1 mL	Lp
13	5246	250	50	acid MF 20 mL	Lp
14	5247	500	100	acid MF 20 mL	Lp
15	5248	55	11	heat MF 20 mL	Lp
16	5249	1200	12	heat combined DP	Lp +L.sp.
17	5250	900	9	heat 1 mL	Lp
18	5251	3400	34	heat 1 mL	Lp
19	5252	1200	12	heat 1 mL	Lp
20	5253	55	11	acid MF 20 mL	Lp
21	5254	250	50	acid MF 20 mL	Lp
22	5255	1600	17	heat combined DP	Lp
23	5256	640	7	heat combined DP	Lp
24	5258	5000	50	heat combined DP	Lp
25	5424	<DL	0		
26	5425	8000	8	acid 0.1 mL	L. sp.
27	5431	200	40	heat MF 20 mL	Lp
28	5470	16000	16	acid 0.1 mL	Lp +L.sp.
29	5471	27000	27	acid 0.1 mL	Lp
30	5473	1000	1	acid 0.1 mL	L. sp.
31	5475	61000	61	acid 0.1 mL	Lp
32	5476	3000	30	heat combined DP	Lp
33	5477	22000	22	acid 0.1 mL	Lp
34	5478	32000	32	acid 0.1 mL	Lp
35	5479	10000	101	heat 1 mL	Lp
36	5480	3900	39	heat 1 mL	Lp
37	5612	5	1	acid MF 20 mL	Lp
38	5613	140	28	acid MF 20 mL	Lp
39	5614	<DL	0		
40	5615	15000	15	acid 0.1 mL	Lp +L.sp.
41	5616	2000	2	no treatm. 0.1 mL	L. sp.
42	5617	<DL	0		
43	5618	<DL	0		
44	5709	<DL	0		
45	5694	16000	157	heat 1 mL	Lp
46	5695	640	128	heat MF 20 mL	Lp
47	5696	4000	4	acid 0.1 mL	Lp
48	5702	1000	1	heat 0.1 mL	Lp
49	5786	120	23	acid MF 20 mL	Lp

Sample Number	Lab ID H2018-	Final result [cfu/100 mL]	Number of cfu for Final Result Calculation	Treatment and Volume for Final Result Calculation	Serology (Latex Test Kit)
50	5813	60	12	acid MF 20 mL	Lp
51	5814	130	26	acid MF 20 mL	Lp
52	5815	700	7	heat 1 mL	Lp +L.sp.
53	5817	<DL	0		
54	5818	1400	14	heat 1 mL	Lp
55	5819	100	1	heat 1 mL	Lp
56	5820	180	36	acid MF 20 mL	Lp
57	5821	110	21	acid MF 20 mL	Lp
58	5822	<DL	0		
59	5823	150	29	acid MF 20 mL	Lp
60	5824	1000	1	heat 0.1 mL	Lp
61	5946	300	3	heat 1 mL	Lp
62	5947	<DL	0		
63	5948	<DL	0		
64	5949	1000	1	acid 0.1 mL	L. sp.
65	5953	<DL	0		
66	5955	<DL	0		
67	5968	<DL	0		
68	5969	110	21	heat MF 20 mL	Lp
69	5986	130	26	acid MF 20 mL	Lp
70	5987	180	35	heat MF 20 mL	Lp
71	5988	170	34	acid MF 20 mL	Lp
72	5989	120	23	acid MF 20 mL	Lp
73	6134	<DL	0		
74	6135	<DL	0		
75	6136	<DL	0		
76	6137	35	7	acid MF 20 mL	Lp
77	6138	<DL	0		
78	6169	100	1	heat 1 mL	Lp
79	6170	15	3	heat MF 20 mL	Lp
80	6180	2900	29	heat 1 mL	Lp
81	6181	150	29	acid MF 20 mL	Lp
82	6223	30	6	acid MF 20 mL	Lp
83	6363	<DL	0		
84	6365	<DL	0		
85	6366	<DL	0		
86	6403	<DL	0		
87	6425	20	4	heat MF 20 mL	L. sp.
88	6426	<DL	0		
89	6448	<DL	0		
90	6449	<DL	0		
91	6462	<DL	0		
92	6477	10000	102	heat 1 mL	Lp
93	6478	2700	27	heat 1 mL	Lp
94	6493	5700	57	heat 1 mL	Lp
95	6798	<DL	0		
96	6799	<DL	0		
97	6800	<DL	0		
98	6802	<DL	0		
99	6803	<DL	0		

DA Table A 3: ISO method - *L. pneumophila* counts.

Sample Number	Lab ID H2018-	Final result [cfu/100 mL]	Number of cfu for Final Result Calculation	Treatment and Volume for Final Result Calculation
1	5084	2500	25	heat 1 mL
2	5091	<DL	0	
3	5092	2000	2	heat 0.1 mL
4	5093	1000	1	heat 0.1 mL
5	5098	4000	4	acid 0.1 mL
6	5099	25	5	acid MF 20 mL
7	5108	1000	1	no treatm. 0.1 mL
8	5111	12000	12	acid 0.1 mL
9	5112	2200	22	heat combined DP
10	5113	24000	239	heat combined DP
11	5114	4800	48	heat 1 mL
12	5115	1800	18	heat 1 mL
13	5246	250	50	acid MF 20 mL
14	5247	500	100	acid MF 20 mL
15	5248	55	11	heat MF 20 mL
16	5249	1000	10	heat 1 mL
17	5250	900	9	heat 1 mL
18	5251	3400	34	heat 1 mL
19	5252	1200	12	heat 1 mL
20	5253	55	11	acid MF 20 mL
21	5254	250	50	acid MF 20 mL
22	5255	1600	17	heat combined DP
23	5256	640	7	heat combined DP
24	5258	5000	50	heat combined DP
25	5424	<DL	0	
26	5425	<DL	0	
27	5431	200	40	heat MF 20 mL
28	5470	12000	12	acid 0.1 mL
29	5471	27000	27	acid 0.1 mL
30	5473	<DL	0	
31	5475	61000	61	acid 0.1 mL
32	5476	3000	30	heat combined DP
33	5477	22000	22	acid 0.1 mL
34	5478	32000	32	acid 0.1 mL
35	5479	10000	101	heat 1 mL
36	5480	3900	39	heat 1 mL
37	5612	5	1	acid MF 20 mL
38	5613	140	28	acid MF 20 mL
39	5614	<DL	0	
40	5615	75	15	acid MF 20 mL
41	5616	<DL	0	
42	5617	<DL	0	
43	5618	<DL	0	
44	5709	<DL	0	
45	5694	16000	157	heat 1 mL
46	5695	640	128	heat MF 20 mL
47	5696	4000	4	acid 0.1 mL
48	5702	1000	1	heat 0.1 mL
49	5786	120	23	acid MF 20 mL
50	5813	60	12	acid MF 20 mL

Sample Number	Lab ID H2018-	Final result [cfu/100 mL]	Number of cfu for Final Result Calculation	Treatment and Volume for Final Result Calculation
51	5814	130	26	acid MF 20 mL
52	5815	600	6	heat 1 mL
53	5817	<DL	0	
54	5818	1400	14	heat 1 mL
55	5819	100	1	heat 1 mL
56	5820	180	36	acid MF 20 mL
57	5821	110	21	acid MF 20 mL
58	5822	<DL	0	
59	5823	150	29	acid MF 20 mL
60	5824	1000	1	heat 0.1 mL
61	5946	300	3	heat 1 mL
62	5947	<DL	0	
63	5948	<DL	0	
64	5949	<DL	0	
65	5953	<DL	0	
66	5955	<DL	0	
67	5968	<DL	0	
68	5969	110	21	heat MF 20 mL
69	5986	130	26	acid MF 20 mL
70	5987	180	35	heat MF 20 mL
71	5988	170	34	acid MF 20 mL
72	5989	120	23	acid MF 20 mL
73	6134	<DL	0	
74	6135	<DL	0	
75	6136	<DL	0	
76	6137	35	7	acid MF 20 mL
77	6138	<DL	0	
78	6169	100	1	heat 1 mL
79	6170	15	3	heat MF 20 mL
80	6180	2900	29	heat 1 mL
81	6181	150	29	acid MF 20 mL
82	6223	30	6	acid MF 20 mL
83	6363	<DL	0	
84	6365	<DL	0	
85	6366	<DL	0	
86	6403	<DL	0	
87	6425	<DL	0	
88	6426	<DL	0	
89	6448	<DL	0	
90	6449	<DL	0	
91	6462	<DL	0	
92	6477	10000	102	heat 1 mL
93	6478	2700	27	heat 1 mL
94	6493	5700	57	heat 1 mL
95	6798	<DL	0	
96	6799	<DL	0	
97	6800	<DL	0	
98	6802	<DL	0	
99	6803	<DL	0	

DA Table A 4: Legiolert.

Sample Number	Lab ID H2018-	Final Result from 1 mL Sample Volume [mpn/100 mL]	Number of Positive Wells	Final Result from 10 mL Sample Volume [mpn/100 mL]	Number of Positive Wells	Maximum Result "Legiolert max."	Best Result "Legiolert best"
1	5084	3100	11	854	20	3100	3100
2	5091	656	5	264	10	656	264
3	5092	1244	7	361	12	1244	361
4	5093	2640	10	1490	29	2640	2640
5	5098	2230	9	310	11	2230	310
6	5099	<DL	0	11	1	11	11
7	5108	843	5	23	2	843	843
8	5111	10573	23	3071	47	10573	10573
9	5112	5957	16	788	19	5957	5957
10	5113	40956	56	TNTC	96	40956	40956
11	5114	5957	16	3614	52	5957	5957
12	5115	2640	10	<DL	0	2640	2640
13	5246	1867	8	<DL	0	1867	1867
14	5247	1038	6	<DL	0	1038	1038
15	5248	1062	7	11	1	1062	1062
16	5249	843	5	<DL	0	843	843
17	5250	1038	6	<DL	0	1038	1038
18	5251	7880	19	11	1	7880	7880
19	5252	3608	12	<DL	0	3608	3608
20	5253	1867	8	361	12	1867	361
21	5254	1244	7	11	1	1244	1244
22	5255	9886	22	416	13	9886	9886
23	5256	4736	14	<DL	0	4736	4736
24	5258	9208	21	<DL	0	9208	9208
25	5424	<DL	0	23	2	23	23
26	5425	<DL	0	<DL	0	0	0
27	5431	1259	6	361	12	1259	361
28	5470	27705	44	10616	85	27705	27705
29	5471	75964	76	22726	95	75964	75964
30	5473	386	3	11	1	386	386
31	5475	49164	62	TNTC	96	49164	49164
32	5476	7229	18	1198	25	7229	7229
33	5477	36142	52	10176	84	36142	36142
34	5478	7880	19	11097	86	11097	11097
35	5479	11270	24	6469	71	11270	11270
36	5480	3100	11	1416	28	3100	3100
37	5612	<DL	0	22	2	22	22
38	5613	386	3	106	7	386	106
39	5614	<DL	0	<DL	0	0	0
40	5615	108	1	90	6	108	90
41	5616	<DL	0	<DL	0	0	0
42	5617	<DL	0	11	1	11	11
43	5618	<DL	0	<DL	0	0	0
44	5709	<DL	0	11	1	11	11
45	5694	15664	30	8128	78	15664	15664
46	5695	1259	6	1198	25	1259	1198
47	5696	4157	13	2048	36	4157	4157

Sample Number	Lab ID H2018-	Final Result from 1 mL Sample Volume [mpn/100 mL]	Number of Positive Wells	Final Result from 10 mL Sample Volume [mpn/100 mL]	Number of Positive Wells	Maximum Result "Legiolert max."	Best Result "Legiolert best"
48	5702	1244	7	169	9	1244	169
49	5786	100	1	104	6	104	104
50	5813	<DL	0	11	1	11	11
51	5814	108	1	106	7	108	106
52	5815	234	2	106	7	234	106
53	5817	108	1	361	12	361	361
54	5818	234	2	474	14	474	474
55	5819	353	3	223	9	353	223
56	5820	<DL	0	39	3	39	39
57	5821	234	2	58	4	234	58
58	5822	<DL	0	11	1	11	11
59	5823	353	3	84	5	353	84
60	5824	896	6	1490	29	1490	1490
61	5946	386	3	66	5	386	66
62	5947	<DL	0	<DL	0	0	0
63	5948	<DL	0	10	1	10	10
64	5949	<DL	0	<DL	0	0	0
65	5953	<DL	0	<DL	0	0	0
66	5955	<DL	0	<DL	0	0	0
67	5968	<DL	0	<DL	0	0	0
68	5969	<DL	0	32	3	32	32
69	5986	234	2	66	5	234	66
70	5987	234	2	39	3	234	39
71	5988	386	3	104	6	386	104
72	5989	<DL	0	169	9	169	169
73	6134	<DL	0	<DL	0	0	0
74	6135	<DL	0	<DL	0	0	0
75	6136	108	1	<DL	0	108	108
76	6137	<DL	0	<DL	0	0	0
77	6138	116296	87	17178	93	116296	116296
78	6169	<DL	0	<DL	0	0	0
79	6170	<DL	0	<DL	0	0	0
80	6180	1462	8	659	17	1462	659
81	6181	234	2	32	3	234	32
82	6223	<DL	0	<DL	0	0	0
83	6363	<DL	0	<DL	0	0	0
84	6365	<DL	0	<DL	0	0	0
85	6366	<DL	0	<DL	0	0	0
86	6403	1259	6	<DL	0	1259	1259
87	6425	<DL	0	11	1	11	11
88	6426	<DL	0	<DL	0	0	0
89	6448	<DL	0	<DL	0	0	0
90	6449	<DL	0	<DL	0	0	0
91	6462	<DL	0	<DL	0	0	0
92	6477	7880	19	3071	47	7880	7880
93	6478	1062	7	534	15	1062	534
94	6493	2640	10	1342	27	2640	2640
95	6798	3100	11	1722	32	3100	3100

Sample Number	Lab ID H2018-	Final Result from 1 mL Sample Volume [mpn/100 mL]	Number of Positive Wells	Final Result from 10 mL Sample Volume [mpn/100 mL]	Number of Positive Wells	Maximum Result "Legiolert max."	Best Result "Legiolert best"
96	6799	<DL	0	<DL	0	0	0
97	6800	<DL	0	<DL	0	0	0
98	6802	<DL	0	<DL	0	0	0
99	6803	<DL	0	<DL	0	0	0

Data for the retrospective analyses (section 3.2)

DA Table B 1: Cooling Tower 1 – Microbiological concentrations from the Laboratory of Technical Hygiene, IHPH.

Legionella Sample #	Sampling Date	Sampling Time	*Legionella* [cfu/100 mL]	Serology	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	HPC Sample #
1	10.01.2012	09:10:00	<DL				
2	31.01.2012	09:00:00	40	2 - 14			
3	06.03.2012	10:00:00	20	2 - 14			
4	03.04.2012	09:00:00	<DL				
5	08.05.2012	12:00:00	<DL				
6	05.06.2012	09:00:00	<DL				
7	10.07.2012	09:10:00	<DL				
8	07.08.2012	08:40:00	50	2 - 14			
9	04.09.2012	09:20:00	<DL				
10	01.10.2012	09:00:00	<DL				
11	06.11.2012	09:10:00	20	2 - 14			
12	04.12.2012	11:10:00	<DL				
13	08.01.2013	08:50:00	<DL				
14	04.02.2013	08:45:00	<DL				
15	05.03.2013	09:20:00	5	2 - 14			
16	02.04.2013	08:40:00	<DL				
17	06.05.2013	08:45:00	25	2 - 14			
18	03.06.2013	11:00:00	90	2 - 14			
19	02.07.2013	08:50:00	<DL				
20	13.08.2013	08:45:00	40	2 - 14			
21	03.09.2013	08:50:00	<DL				
22	24.09.2013	08:40:00	<DL				
23	05.11.2013	08:35:00	<DL				
24	03.12.2013	12:45:00	25	2 - 14			
25	14.01.2014	09:40:00	<DL				
26	11.02.2014	08:50:00	<DL				
27	11.03.2014	11:00:00	130	2 - 14			
28	08.04.2014	09:00:00	10	2 - 14			
29	06.05.2014	10:00:00	610	1, 2 - 14			
30	10.06.2014	11:00:00	50	1			
31	08.07.2014	12:00:00	<DL				
32	05.08.2014	11:35:00	<DL				
33	02.09.2014	08:45:00	10	2 - 14			
34	07.10.2014	09:40:00	<DL				
35	04.11.2014	12:40:00	40	2 - 14			
36	02.12.2014	11:00:00	515	1, 2 - 14			
37	13.01.2015	10:10:00	<DL				
38	10.02.2015	10:10:00	<DL				
39	10.03.2015	10:00:00	<DL				
40	07.04.2015	09:20:00	30	2 - 14			
41	05.05.2015	12:20:00	45	1, 2 - 14			
42	02.06.2015	09:40:00	105				
43	07.07.2015	10:10:00	<DL				
44	04.08.2015	08:50:00	80	2 - 14			

Legionella Sample #	Sampling Date	Sampling Time	*Legionella* [cfu/100 mL]	Serology	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	HPC Sample #
45	16.09.2015	08:40:00	20	2 - 14			
46	06.10.2015	13:30:00	<DL				
47	17.11.2015	10:50:00	35	2 - 14			
48	01.12.2015	10:10:00	60	2 - 14			
49	12.01.2016	10:20:00	500	1, 2 - 14			
50	02.02.2016	08:55:00	<DL				
51	07.03.2016	10:20:00	20	L. spec			
52	06.04.2016	08:40:00	5	2 - 14			
53	11.05.2016	08:50:00	<DL				
54	07.06.2016	09:07:00	20	2 - 14			
55	04.07.2016	08:47:00	15	2 - 14			
56	02.08.2016	16:30:00	10	2 - 14			
57	06.09.2016	09:11:00	65	2 - 14			
58	04.10.2016	10:12:00	35	2 - 14			
59	02.11.2016	10:06:00	100	2 - 14			
60	06.12.2016	09:24:00	40	2 - 14			
61	03.01.2017	10:03:00	25	2 - 14	1620	2160	1
62	07.02.2017	09:12:00	105	2 - 14	169	240	2
63	07.03.2017	09:08:00	50	2 - 14	285	126	3
64	04.04.2017	09:36:00	5		470	420	4
65	02.05.2017	09:37:00	10	2 - 14	780	1080	5
66	06.06.2017	08:39:00	30	2 - 14			
67	03.07.2017	09:24:00	10	2 - 14	16400	11800	6
68	01.08.2017	09:45:00	115	2 - 14	600	450	7
69	05.09.2017	09:02:00	500	2 - 14	1300	1690	8
70	04.10.2017	09:55:00	45	2 - 14	68	160	9
71	07.11.2017	10:22:00	20	2 - 14	320	124	10
72	05.12.2017	09:53:00	5	2 - 14	1	5	11
73	09.01.2018	10:18:00	60	2 - 14	590	250	12
74	06.02.2018	10:16:00	15	2 - 14	220	60	13
75	06.03.2018	09:47:00	<DL		2800	1290	14
76	10.04.2018	08:00:00	55	1, 2 - 14	660	130	15
77	08.05.2018	08:45:00	25	2 - 14	1800	1800	16
78	05.06.2018	09:37:00	<DL		20	540	17
79	03.07.2018	10:13:00	<DL		1580	1940	18
80	07.08.2018	09:33:00	15	2 - 14	950	280	19
81	04.09.2018	11:40:00	40	2 - 14	10	10	20
82	01.10.2018	10:55:00	70	2 - 14	270	270	21
83	05.11.2018	08:56:00	<DL		100	60	22
84	03.12.2018	08:38:00	15	2 - 14	1180	980	23
	07.01.2019	08:59:00	45		210	80	24
	04.02.2019	09:17:00	<DL				
	05.03.2019	08:31:00	10		30	10	25
	01.04.2019	10:09:00	10		350	30	26
	06.05.2019	09:37:00	5		840	280	27
	03.06.2019	09:41:00	<DL		200	150	28
	01.07.2019	09:04:00	10		120	10	29
	05.08.2019	08:25:00	<DL		5100	1800	30

Legionella Sample #	Sampling Date	Sampling Time	*Legionella* [cfu/100 mL]	Serology	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	HPC Sample #
	02.09.2019	09:49:00	<DL		<DL	<DL	31
	07.10.2019	09:34:00	5		490	270	
	07.10.2019	10:45:00	20		350	40	32
	14.10.2019	08:20:00	40		180	30	33
	21.10.2019	07:45:00	120		320	190	34
	28.10.2019	10:05:00	250		290	40	35
	04.11.2019	09:41:00	330		280	470	36
	11.11.2019	10:15:00	200		480	120	37
	18.11.2019	08:45:00	270		320	100	38
	25.11.2019	08:30:00	<DL		30240	73200	39
	02.12.2019	09:17:00	<DL		230	620	40
	16.12.2019	09:00:00	90		1800	1110	41
	06.01.2020	10:17:00	35		100	160	
	03.02.2020	10:22:00	<DL		50	50	
	02.03.2020	09:56:00	10		960	130	
	06.04.2020	09:10:00	20		280	630	
	04.05.2020	09:36:00	<DL		80	10	
	02.06.2020	09:43:00	65		130000	3000	
	06.07.2020	09:26:00	<DL		140	40	
	03.08.2020	09:23:00	45		540	210	
	07.09.2020	09:45:00	5		260	20	

DA Table B 2: Cooling Tower 1 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 5 days.

Sample #	Biocide dosages 5 days before sampling	Biocide dosages 5 days after sampling	Biocide dosages 5 days before and after sampling	Ratio of dosages 5 days after and before sampling	Mean mV 5 days before sampling	Time [h] below threshold 5 days before sampling
1	34	22	56	0.647058824	411.894	74
2	42	52	94	1.238095238	452.0076471	51
3	11	37	48	3.363636364	408.9470588	72
4	17	23	40	1.352941176	489.3464706	12
5	16	18	34	1.125	467.2294118	9
6	21	21	42	1	490.6329412	2
7	22	11	33	0.5	477.0435833	4
8	19	7	26	0.368421053	487.0185833	5
9	8	41	49	5.125	364.28875	74
10	42	30	72	0.714285714	560.7452941	15
11	17	18	35	1.058823529	442.9681667	60
12	11	47	58	4.272727273	305.1784167	99
13	39	44	83	1.128205128	552.0151919	9
14	48	27	75	0.5625	533.4874118	23
15	22	43	65	1.954545455	412.2163147	74
16	23	25	48	1.086956522	372.5051913	80
17	20	17	37	0.85	463.7416631	13
18	16	16	32	1	470.3166401	26
19	24	15	39	0.625	504.7302539	21
20	18	9	27	0.5	475.8565587	10
21	29	22	51	0.75862069	416.4902064	58
22	16	15	31	0.9375	481.865299	22
23	18	18	36	1	503.7724332	2
24	18	15	33	0.833333333	508.4294607	6
25	16	16	32	1	517.2304558	7
26	17	11	28	0.647058824	472.068791	13
27	18	25	43	1.388888889	473.0489615	15
28	18	14	32	0.777777778	467.0243525	22
29	12	15	27	1.25	459.4843514	17
30	18	15	33	0.833333333	452.9696676	23
31	21	15	36	0.714285714	467.3227131	20
32	49	47	96	0.959183673	470.3793119	41
33	47	44	91	0.936170213	504.6110367	32
34	18	17	35	0.944444444	495.4537415	2
35	22	19	41	0.863636364	478.5930064	18
36	3	14	17	4.666666667	384.9208833	108
37	15	17	32	1.133333333	507.1117729	14
38	14	17	31	1.214285714	483.5071073	35
39	20	16	36	0.8	495.4181385	18
40	18	16	34	0.888888889	501.2999761	18
41	18	20	38	1.111111111	485.5628363	17
42	20	17	37	0.85	531.4523631	21
43	45	11	56	0.244444444	394.4867485	66
44	34	30	64	0.882352941	361.881562	74
45	12	9	21	0.75	506.3703046	7
46	16	14	30	0.875	474.2484884	16
47	14	15	29	1.071428571	471.6454107	9

Sample #	Biocide dosages 5 days before sampling	Biocide dosages 5 days after sampling	Biocide dosages 5 days before and after sampling	Ratio of dosages 5 days after and before sampling	Mean mV 5 days before sampling	Time [h] below threshold 5 days before sampling
48	10	10	20	1	504.2324036	9
49	25	17	42	0.68	473.5358251	28
50	12	10	22	0.833333333	457.5295558	21
51	15	17	32	1.133333333	431.8806742	39
52	20	23	43	1.15	438.6318385	10
53	19	22	41	1.157894737	440.5050008	21
54	13	14	27	1.076923077	419.3379484	58
55	9	11	20	1.222222222	475.7300387	8
56	16	15	31	0.9375	437.8785568	22
57	14	15	29	1.071428571	494.2793732	18
58	8	19	27	2.375	435.944208	36
59	12	13	25	1.083333333	497.5809979	13
60	11	17	28	1.545454545	483.5551109	5
61	10	13	23	1.3	482.5988968	13
62	1	10	11	10	352.1207041	118
63	15	17	32	1.133333333	458.6081449	21
64	14	13	27	0.928571429	487.3290883	16
65	9	10	19	1.111111111	463.7449158	13
66	14	10	24	0.714285714	491.2835836	11
67	13	13	26	1	468.4994576	15
68	10	11	21	1.1	475.0710897	11
69	8	8	16	1	477.9045942	5
70	8	9	17	1.125	494.3738323	6
71	0	0	0	0	553.2925451	0
72	0	2	2	2	459.3930384	0
73	10	6	16	0.6	488.7939184	15
74	16	10	26	0.625	493.5297719	6
75	12	17	29	1.416666667	491.5849899	8
76	17	18	35	1.058823529	428.8560723	35
77	13	15	28	1.153846154	432.815358	4
78	14	17	31	1.214285714	435.4081866	4
79	19	17	36	0.894736842	441.409995	25
80	20	15	35	0.75	411.8816116	45
81	0	18	18	18	29.95030346	20
82	12	10	22	0.833333333	441.2144939	20
83	32	9	41	0.28125	464.2572563	41
84	14	18	32	1.285714286	496.4467	13

DA Table B 3: Cooling Tower 1 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 5 days.

Sample #	Biocide dosages 5 days before sampling	Biocide dosages 5 days after sampling	Biocide dosages 5 days before and after sampling	Ratio of dosages 5 days after and before sampling	Mean mV 5 days before sampling	Time [h] below threshold 5 days before sampling
1	10	13	23	1.3	482.5988968	13
2	1	10	11	10	352.1207041	118
3	15	17	32	1.133333333	458.6081449	21
4	14	13	27	0.928571429	487.3290883	16
5	9	10	19	1.111111111	463.7449158	13
6	13	13	26	1	468.4994576	15
7	10	11	21	1.1	475.0710897	11
8	8	8	16	1	477.9045942	5
9	8	9	17	1.125	494.3738323	6
10	0	0	0	0	553.2925451	0
11	0	2	2	2	459.3930384	0
12	10	6	16	0.6	488.7939184	15
13	16	10	26	0.625	493.5297719	6
14	12	17	29	1.416666667	491.5849899	8
15	17	18	35	1.058823529	428.8560723	35
16	13	15	28	1.153846154	432.815358	4
17	14	17	31	1.214285714	435.4081866	4
18	19	17	36	0.894736842	441.409995	25
19	20	15	35	0.75	411.8816116	45
20	0	18	18	18	29.95030346	20
21	12	10	22	0.833333333	441.2144939	20
22	32	9	41	0.28125	464.2572563	41
23	14	18	32	1.285714286	496.4467	13
24	11	12	23	1.090909091	467.1661402	6
25	15	14	29	0.933333333	458.0640345	14
26	11	9	20	0.818181818	460.9752136	4
27	11	8	19	0.727272727	472.6075139	5
28	14	12	26	0.857142857	457.3481415	11
29	14	0	14	0	480.5530406	14
30	14	0	14	0	491.6207855	11
31	10	0	10	0	470.7398748	15
32	11	0	11	0	463.6510778	12
33	2	0	2	0	331.8287884	114
34	0	0	0	0	365.9747777	120
35	4	0	4	0	380.039233	88
36	11	0	11	0	401.6554924	42
37	0	0	0	0	312.5807724	120
38	0	0	0	0	366.3536547	120
39	7	0	7	0	370.1098043	86
40	17	0	17	0	454.53815	1
41	13	0	13	0	457.4247272	19

DA Table B 4: Cooling Tower 1 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 7 days.

Sample #	Biocide dosages 7 days before sampling	Biocide dosages 7 days after sampling	Biocide dosages 7 days before and after sampling	Ratio of dosages 7 days after and before sampling	Mean mV 7 days before sampling	Time [h] below threshold 7 days before sampling
1	54	31	85	0.574074074	431.08	88
2	63	69	132	1.095238095	460.5442515	70
3	17	58	75	3.411764706	414.060479	90
4	25	31	56	1.24	494.4447904	14
5	25	25	50	1	469.5750898	12
6	30	29	59	0.966666667	496.128982	2
7	32	16	48	0.5	483.69875	4
8	27	7	34	0.259259259	487.78875	6
9	18	58	76	3.222222222	382.75125	100
10	58	48	106	0.827586207	564.2423353	23
11	24	26	50	1.083333333	425.7108333	94
12	13	62	75	4.769230769	288.4316667	144
13	46	65	111	1.413043478	485.3822622	45
14	56	47	103	0.839285714	480.573818	59
15	31	57	88	1.838709677	409.4770303	105
16	35	39	74	1.114285714	379.492836	110
17	27	23	50	0.851851852	466.9543301	17
18	23	23	46	1	476.851955	31
19	30	21	51	0.7	499.3162013	25
20	25	9	34	0.36	474.9123708	14
21	38	29	67	0.763157895	438.2366102	64
22	22	21	43	0.954545455	477.5788718	36
23	25	25	50	1	500.2936823	2
24	25	21	46	0.84	514.415317	8
25	22	22	44	1	518.0356765	7
26	26	14	40	0.538461538	467.4609423	18
27	29	33	62	1.137931034	456.7926151	28
28	27	21	48	0.777777778	471.2836954	31
29	17	20	37	1.176470588	458.1189466	25
30	23	19	42	0.826086957	453.3309726	31
31	26	21	47	0.807692308	467.9807873	28
32	57	65	122	1.140350877	472.3852852	51
33	66	63	129	0.954545455	497.8022775	47
34	24	23	47	0.958333333	494.7873443	8
35	28	27	55	0.964285714	493.0878445	24
36	11	21	32	1.909090909	391.8424111	124
37	21	24	45	1.142857143	513.8416281	20
38	21	23	44	1.095238095	487.9113948	42
39	27	22	49	0.814814815	498.1938069	23
40	24	23	47	0.958333333	500.958614	26
41	24	28	52	1.166666667	489.0148377	23
42	29	22	51	0.75862069	540.6545768	29
43	65	15	80	0.230769231	390.0885542	93
44	54	48	102	0.888888889	375.3780289	95
45	16	13	29	0.8125	495.2888165	12
46	22	20	42	0.909090909	489.1249677	20
47	22	19	41	0.863636364	466.227674	12

Sample #	Biocide dosages 7 days before sampling	Biocide dosages 7 days after sampling	Biocide dosages 7 days before and after sampling	Ratio of dosages 7 days after and before sampling	Mean mV 7 days before sampling	Time [h] below threshold 7 days before sampling
48	16	15	31	0.9375	500.1496822	12
49	32	23	55	0.71875	436.9541815	68
50	14	15	29	1.071428571	433.2876591	57
51	23	27	50	1.173913043	440.3721635	48
52	23	29	52	1.260869565	449.5404699	15
53	25	29	54	1.16	451.3770968	27
54	23	18	41	0.782608696	439.5490267	65
55	13	18	31	1.384615385	473.3075447	13
56	23	23	46	1	442.7138608	31
57	21	22	43	1.047619048	493.9450947	26
58	14	24	38	1.714285714	437.8441369	46
59	16	15	31	0.9375	494.518477	16
60	14	21	35	1.5	481.8913988	5
61	15	19	34	1.266666667	484.7010467	20
62	2	16	18	8	360.7805975	156
63	23	26	49	1.130434783	455.4749293	38
64	21	20	41	0.952380952	483.9601649	22
65	12	14	26	1.166666667	450.9195647	19
66	20	14	34	0.7	489.0898443	17
67	17	20	37	1.176470588	470.1493421	22
68	15	15	30	1	474.4622587	18
69	13	14	27	1.076923077	474.2784547	13
70	11	12	23	1.090909091	488.098032	8
71	0	0	0	0	556.6510965	0
72	0	5	5	5	462.75159	0
73	15	6	21	0.4	487.9918149	19
74	25	14	39	0.56	485.0805477	11
75	16	23	39	1.4375	486.9775823	10
76	24	28	52	1.166666667	437.1298078	40
77	22	19	41	0.863636364	427.8449807	8
78	19	25	44	1.315789474	443.0645723	8
79	26	24	50	0.923076923	442.4645794	34
80	29	21	50	0.724137931	422.3595305	51
81	0	28	28	28	207.0083027	54
82	16	17	33	1.0625	448.9756243	20
83	49	21	70	0.428571429	470.5462824	55
84	25	24	49	0.96	504.2878913	21

DA Table B 5: Cooling Tower 1 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 7 days.

Sample #	Biocide dosages 7 days before sampling	Biocide dosages 7 days after sampling	Biocide dosages 7 days before and after sampling	Ratio of dosages 7 days after and before sampling	Mean mV 7 days before sampling	Time [h] below threshold 7 days before sampling
1	15	19	34	1.266666667	484.7010467	20
2	2	16	18	8	360.7805975	156
3	23	26	49	1.130434783	455.4749293	38
4	21	20	41	0.952380952	483.9601649	22
5	12	14	26	1.166666667	450.9195647	19
6	17	20	37	1.176470588	470.1493421	22
7	15	15	30	1	474.4622587	18
8	13	14	27	1.076923077	474.2784547	13
9	11	12	23	1.090909091	488.098032	8
10	0	0	0	0	556.6510965	0
11	0	5	5	5	462.75159	0
12	15	6	21	0.4	487.9918149	19
13	25	14	39	0.56	485.0805477	11
14	16	23	39	1.4375	486.9775823	10
15	24	28	52	1.166666667	437.1298078	40
16	22	19	41	0.863636364	427.8449807	8
17	19	25	44	1.315789474	443.0645723	8
18	26	24	50	0.923076923	442.4645794	34
19	29	21	50	0.724137931	422.3595305	51
20	0	28	28	28	207.0083027	54
21	16	17	33	1.0625	448.9756243	20
22	49	21	70	0.428571429	470.5462824	55
23	25	24	49	0.96	504.2878913	21
24	17	16	33	0.941176471	465.1396588	11
25	22	18	40	0.818181818	452.88332	16
26	14	14	28	1	466.8054881	5
27	14	12	26	0.857142857	471.9158238	7
28	17	17	34	1	464.1074524	14
29	20	0	20	0	469.7687303	24
30	23	0	23	0	482.965907	20
31	18	0	18	0	475.5853464	23
32	14	0	14	0	455.5105465	33
33	9	0	9	0	363.2506937	114
34	0	0	0	0	367.9504142	168
35	4	0	4	0	372.5285536	136
36	12	0	12	0	400.4216656	82
37	0	0	0	0	313.8265526	167
38	0	0	0	0	359.285376	168
39	12	0	12	0	376.1990221	111
40	26	0	26	0	450.6836059	4
41	18	0	18	0	455.5619074	29

DA Table B 6: Cooling Tower 1 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 10 days.

Sample #	Biocide dosages 10 days before sampling	Biocide dosages 10 days after sampling	Biocide dosages 10 days before and after sampling	Ratio of dosages 10 days after and before sampling	Mean mV 10 days before sampling	Time [h] below threshold 10 days before sampling
1	65	64	129	0.984615385	413.7896	131
2	93	97	190	1.043010753	479.4338075	88
3	25	90	115	3.6	417.5087029	118
4	37	37	74	1	498.14	16
5	35	33	68	0.942857143	465.5676569	19
6	47	41	88	0.872340426	493.6329707	3
7	48	22	70	0.458333333	488.3447917	4
8	41	15	56	0.365853659	488.7647917	9
9	45	81	126	1.8	439.4462917	112
10	83	75	158	0.903614458	567.715523	29
11	37	36	73	0.972972973	432.2372199	124
12	23	75	98	3.260869565	301.8047083	203
13	70	78	148	1.114285714	455.9342468	82
14	65	75	140	1.153846154	434.6379583	120
15	44	75	119	1.704545455	410.7884282	148
16	54	56	110	1.037037037	389.3273826	151
17	39	32	71	0.820512821	469.4489324	29
18	32	32	64	1	476.4951914	50
19	39	30	69	0.769230769	495.9616915	35
20	45	20	65	0.444444444	477.1777793	25
21	47	38	85	0.808510638	438.4130816	97
22	32	30	62	0.9375	473.8176645	52
23	36	35	71	0.972222222	489.0917054	2
24	34	31	65	0.911764706	517.4916209	9
25	33	32	65	0.96969697	512.4313442	11
26	32	24	56	0.75	445.0346393	67
27	52	41	93	0.788461538	436.3897072	66
28	37	28	65	0.756756757	472.1994756	40
29	27	28	55	1.037037037	452.1508014	41
30	33	26	59	0.787878788	454.7296292	40
31	36	32	68	0.888888889	471.6987904	38
32	72	91	163	1.263888889	477.2739288	65
33	96	85	181	0.885416667	490.032872	71
34	33	33	66	1	487.1644653	15
35	37	36	73	0.972972973	491.5617978	26
36	25	31	56	1.24	403.3081267	129
37	30	34	64	1.133333333	517.1832528	29
38	31	32	63	1.032258065	489.0621508	50
39	38	35	73	0.921052632	495.5553396	32
40	36	32	68	0.888888889	487.2975691	41
41	34	41	75	1.205882353	490.0687757	33
42	42	30	72	0.714285714	536.1383111	40
43	80	25	105	0.3125	402.1041453	125
44	88	55	143	0.625	386.8425288	126
45	22	19	41	0.863636364	483.9833538	22
46	27	27	54	1	502.5210392	24

Sample #	Biocide dosages 10 days before sampling	Biocide dosages 10 days after sampling	Biocide dosages 10 days before and after sampling	Ratio of dosages 10 days after and before sampling	Mean mV 10 days before sampling	Time [h] below threshold 10 days before sampling
47	40	27	67	0.675	460.8729833	17
48	21	31	52	1.476190476	491.108234	17
49	39	28	67	0.717948718	453.9135995	74
50	19	24	43	1.263157895	438.3919498	69
51	33	37	70	1.121212121	444.4656274	54
52	31	38	69	1.225806452	457.9044203	20
53	34	38	72	1.117647059	459.7376439	40
54	36	23	59	0.638888889	442.5713411	82
55	23	24	47	1.043478261	468.7137976	22
56	31	29	60	0.935483871	429.3357825	79
57	33	30	63	0.909090909	493.2488853	37
58	25	27	52	1.08	433.9432371	58
59	21	18	39	0.857142857	491.4945788	20
60	18	26	44	1.444444444	479.5333112	6
61	22	27	49	1.227272727	486.3269989	27
62	4	26	30	6.5	372.2488735	210
63	32	38	70	1.1875	456.7668772	48
64	28	27	55	0.964285714	481.0631264	29
65	14	20	34	1.428571429	427.2906386	82
66	34	20	54	0.588235294	474.4518111	38
67	27	29	56	1.074074074	468.3858598	33
68	25	20	45	0.8	475.2625234	27
69	24	21	45	0.875	469.5943437	28
70	16	17	33	1.0625	486.0530166	11
71	1	0	1	0	561.7588942	0
72	0	14	14	14	467.7894175	0
73	26	15	41	0.576923077	486.1374271	23
74	41	23	64	0.56097561	475.8338149	18
75	21	30	51	1.428571429	485.0438171	12
76	33	38	71	1.151515152	445.8242311	50
77	36	24	60	0.666666667	425.0723289	13
78	27	34	61	1.259259259	449.9124378	15
79	44	37	81	0.840909091	441.5124195	37
80	44	32	76	0.727272727	432.0649422	62
81	9	41	50	4.555555556	350.1319628	70
82	26	18	44	0.692307692	454.3049781	20
83	61	30	91	0.491803279	468.0906447	61
84	55	29	84	0.527272727	500.7331798	47

DA Table B 7: Cooling Tower 1 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 10 days.

Sample #	Biocide dosages 10 days before sampling	Biocide dosages 10 days after sampling	Biocide dosages 10 days before and after sampling	Ratio of dosages 10 days after and before sampling	Mean mV 10 days before sampling	Time [h] below threshold 10 days before sampling
1	22	27	49	1.227272727	486.3269989	27
2	4	26	30	6.5	372.2488735	210
3	32	38	70	1.1875	456.7668772	48
4	28	27	55	0.964285714	481.0631264	29
5	14	20	34	1.428571429	427.2906386	82
6	27	29	56	1.074074074	468.3858598	33
7	25	20	45	0.8	475.2625234	27
8	24	21	45	0.875	469.5943437	28
9	16	17	33	1.0625	486.0530166	11
10	1	0	1	0	561.7588942	0
11	0	14	14	14	467.7894175	0
12	26	15	41	0.576923077	486.1374271	23
13	41	23	64	0.56097561	475.8338149	18
14	21	30	51	1.428571429	485.0438171	12
15	33	38	71	1.151515152	445.8242311	50
16	36	24	60	0.666666667	425.0723289	13
17	27	34	61	1.259259259	449.9124378	15
18	44	37	81	0.840909091	441.5124195	37
19	44	32	76	0.727272727	432.0649422	62
20	9	41	50	4.555555556	350.1319628	70
21	26	18	44	0.692307692	454.3049781	20
22	61	30	91	0.491803279	468.0906447	61
23	55	29	84	0.527272727	500.7331798	47
24	25	24	49	0.96	468.0633298	20
25	30	24	54	0.8	451.4384519	19
26	20	19	39	0.95	467.6784522	8
27	20	17	37	0.85	474.9987208	12
28	23	24	47	1.043478261	462.1043896	25
29	26	29	55	1.115384615	469.8224527	40
30	37	33	70	0.891891892	470.1326578	33
31	33	21	54	0.636363636	477.2352219	35
32	23	9	32	0.391304348	462.878046	44
33	16	0	16	0	392.2390769	118
34	2	8	10	4	358.7278543	235
35	4	12	16	3	367.810213	208
36	16	0	16	0	399.9063428	122
37	7	9	16	1.285714286	340.2526117	195
38	0	24	24	24	345.1222286	240
39	12	0	12	0	376.1990221	111
40	26	0	26	0	450.6836059	4
41	18	0	18	0	455.5619074	29

DA Table B 8: Cooling Tower 1 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 5 days

Sample #	Mean water temp. [°C] 5 days before sampling	Min. water temp. [°C] 5 days before sampling	Max. water temp. [°C] 5 days before sampling	Time [h] above threshold 5 days before sampling
1	17.3008	16.354	18.5905	0
2	16.79475	15.1625	17.96	0
3	17.729775	16.2125	19.0155	0
4	16.5207	13.2505	18.4935	0
5	17.21081579	16.075	18.834	0
6	17.378175	15.84	20.1385	6
7	19.7561	17.6095	22.9075	42
8	18.85655	16.6165	21.0385	6
9	17.416975	16.329	18.6	0
10	17.684725	16.2755	18.546	0
11	17.636325	16.079	19.003	0
12	16.750425	15.777	18.4625	0
13	17.358775	16.031	18.423	0
14	17.48555	15.733	18.914	0
15	17.323325	16.4455	18.4985	0
16	17.262125	15.901	18.8115	0
17	17.93095	16.9515	19.411	0
18	17.494875	15.955	19.192	0
19	18.00135	16.0925	19.04	0
20	18.657925	16.5405	20.335	24
21	18.385975	16.4585	19.69	0
22	18.026625	16.485	19.5705	0
23	17.482125	16.448	18.9125	0
24	17.49415	15.993	19.1915	0
25	17.615625	15.613	18.892	0
26	17.822575	15.7875	19.074	0
27	17.67731579	16.4375	18.65	0
28	17.644275	16.5465	18.795	0
29	17.58	15.616	19.2035	0
30	20.323175	16.9565	23.46	66
31	20.04086842	17.8035	22.627	66
32	20.60845	18.6905	22.0375	90
33	18.04915	15.652	19.9415	0
34	17.98775	16.363	19.2085	0
35	18.015925	16.528	19.289	0
36	17.78971053	16.396	18.9025	0
37	17.495625	16.5325	18.607	0
38	17.84735	16.0845	19.345	0
39	17.444125	15.955	19.5235	0
40	17.166175	16.0005	19.0225	0
41	17.78530556	16.255	19.695	0
42	17.789775	16.8905	19.0375	0
43	21.7439	17.28	24.736	84
44	18.965675	16.858	22.5525	18
45	17.647475	14.7025	19.048	0
46	17.9587	16.9125	18.9965	0

Sample #	Mean water temp. [°C] 5 days before sampling	Min. water temp. [°C] 5 days before sampling	Max. water temp. [°C] 5 days before sampling	Time [h] above threshold 5 days before sampling
47	17.8416	16.604	18.847	0
48	17.228425	16.101	18.416	0
49	17.389575	16.2705	18.7005	0
50	17.51395	16.187	18.9285	0
51	15.85535	14.3015	17.948	0
52	16.6367	15.4535	18.63	0
53	17.21835	15.427	18.6425	0
54	20.05685	18.4685	21.216	60
55	17.5691	15.8	19.5625	0
56	18.410425	15.744	20.998	12
57	18.299475	16.2565	20.008	6
58	16.708525	15.2195	18.3095	0
59	16.5427	15.2565	17.9915	0
60	16.490425	15.2575	17.8705	0
61	16.74928788	14.7905	17.855	0
62	16.596425	15.073	17.67	0
63	16.57695	14.891	18.6165	0
64	16.66625	14.7115	18.3865	0
65	16.4331625	14.442	17.9965	0
66	18.732125	15.325	21.4845	34
67	20.0849125	16.2195	22.751	63
68	20.92294167	19.271	22.8785	98
69	18.52652083	16.007	20.8815	21
70	18.79860169	15.397	23.6715	19
71	19.64392917	19.575	19.7125	0
72	18.86610833	18.797	18.935	0
73	17.26301667	15.085	18.863	0
74	15.41969167	13.2645	16.3995	0
75	15.691475	14.485	16.8015	0
76	16.18776891	14.431	18.3945	0
77	16.2798	14.93	18.906	0
78	20.55455417	17.8235	23.547	68
79	17.6148375	14.7905	20.9805	14
80	21.69163333	18.249	24.333	106
81	18.61742917	16.127	21.7875	24
82	15.99397083	14.66	17.1825	0
83	15.93319583	14.842	16.905	0
84	16.380925	15.1405	18.051	0

DA Table B 9: Cooling Tower 1 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 5 days

Sample #	Mean water temp. [°C] 5 days before sampling	Min. water temp. [°C] 5 days before sampling	Max. water temp. [°C] 5 days before sampling	Time [h] above threshold 5 days before sampling
1	16.74928788	14.7905	17.855	0
2	16.596425	15.073	17.67	0
3	16.57695	14.891	18.6165	0
4	16.66625	14.7115	18.3865	0
5	16.4331625	14.442	17.9965	0
6	20.0849125	16.2195	22.751	63
7	20.92294167	19.271	22.8785	98
8	18.52652083	16.007	20.8815	21
9	18.79860169	15.397	23.6715	19
10	19.64392917	19.575	19.7125	0
11	18.86610833	18.797	18.935	0
12	17.26301667	15.085	18.863	0
13	15.41969167	13.2645	16.3995	0
14	15.691475	14.485	16.8015	0
15	16.18776891	14.431	18.3945	0
16	16.2798	14.93	18.906	0
17	20.55455417	17.8235	23.547	68
18	17.6148375	14.7905	20.9805	14
19	21.69163333	18.249	24.333	106
20	18.61742917	16.127	21.7875	24
21	15.99397083	14.66	17.1825	0
22	15.93319583	14.842	16.905	0
23	16.380925	15.1405	18.051	0
24				

DA Table B 10: Cooling Tower 1 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 7 days.

Sample #	Mean water temp. [°C] 7 days before sampling	Min. water temp. [°C] 7 days before sampling	Max. water temp. [°C] 7 days before sampling	Time [h] above threshold 7 days before sampling
1	17.21539286	16.354	18.5905	0
2	16.94642857	15.1625	18.3615	0
3	17.84491071	16.2125	19.0155	0
4	16.77058929	13.2505	18.4935	0
5	17.68009259	16.075	20.7165	6
6	17.66585714	15.84	20.1385	6
7	20.01066071	17.6095	22.9325	66
8	18.99976786	16.6165	21.086	24
9	17.75067857	16.329	19.9655	0
10	17.80685714	16.2755	18.658	0
11	17.41583929	16.079	19.003	0
12	16.75755357	15.676	18.4625	0
13	17.33964286	16.031	18.423	0
14	17.59051786	15.733	18.9455	0
15	17.58633929	16.4455	20.245	6
16	17.11328571	14.646	18.8115	0
17	17.67575	16.23	19.411	0
18	17.58526786	15.955	19.192	0
19	18.00032143	16.0925	19.04	0
20	18.956375	16.5405	22.2245	36
21	18.34907143	16.4585	19.69	0
22	17.98496429	16.485	19.5705	0
23	17.351125	16.2695	18.9125	0
24	17.48157143	15.992	19.1915	0
25	17.537375	15.613	18.892	0
26	17.675875	15.7875	19.074	0
27	17.76464815	16.4375	19.01	0
28	17.65680357	16.5465	18.795	0
29	17.68433929	15.616	19.215	0
30	19.78955357	16.9565	23.46	66
31	19.33381481	16.613	22.627	66
32	20.76794643	18.4755	23.5415	132
33	18.04310714	15.652	19.9415	0
34	18.03592857	16.363	19.2085	0
35	17.88264286	16.528	19.289	0
36	17.63677778	16.396	18.9025	0
37	17.45046429	15.8255	18.703	0
38	17.71083929	16.0845	19.345	0
39	17.437	15.955	19.5235	0
40	17.302625	15.8475	19.083	0
41	17.76905769	16.206	19.695	0
42	17.63919643	16.607	19.0375	0
43	21.26019643	17.28	24.736	102
44	18.69264286	16.858	22.5525	18
45	17.61221429	14.7025	19.048	0
46	17.86075	16.615	18.9965	0

Sample #	Mean water temp. [°C] 7 days before sampling	Min. water temp. [°C] 7 days before sampling	Max. water temp. [°C] 7 days before sampling	Time [h] above threshold 7 days before sampling
47	17.89489286	16.604	18.847	0
48	17.38789286	16.101	19.1115	0
49	17.51694643	16.2705	18.897	0
50	17.62644643	16.187	18.9285	0
51	15.83930357	14.3015	17.948	0
52	16.41114286	15.446	18.63	0
53	16.98435714	15.427	18.6425	0
54	19.83269643	17.43	21.6065	78
55	17.57175	15.8	19.5625	0
56	18.672	15.744	20.998	18
57	17.98544643	15.517	20.008	6
58	16.91180357	15.2195	18.3095	0
59	16.72671429	15.2565	18.0525	0
60	16.52375	15.2575	18.3795	0
61	16.73225	14.7905	17.855	0
62	16.48972917	15.073	17.67	0
63	16.59283036	14.891	18.6165	0
64	16.6840625	14.7115	18.3865	0
65	16.43900893	14.442	17.9965	0
66	18.71469345	15.325	21.5615	46
67	20.15209524	16.2195	22.751	89
68	21.06463393	19.271	22.8785	145
69	19.51587202	16.007	23.678	63
70	19.7430241	15.397	23.6715	67
71	19.67175	19.575	19.7685	0
72	18.89392857	18.797	18.9905	0
73	17.29922321	15.085	18.863	0
74	15.51952976	13.2645	17.386	0
75	15.60387202	14.276	16.8015	0
76	16.18684731	14.431	18.3945	0
77	16.23176488	14.93	18.906	0
78	20.99294643	17.8235	23.6485	116
79	17.6847619	14.7905	20.9805	18
80	21.59413095	18.249	24.333	148
81	18.87680357	16.127	21.7875	37
82	16.10402679	14.66	17.401	0
83	16.15078571	14.842	17.462	0
84	16.3927619	15.0395	18.051	0

DA Table B 11: Cooling Tower 1 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 7 days.

Sample #	Mean water temp. [°C] 7 days before sampling	Min. water temp. [°C] 7 days before sampling	Max. water temp. [°C] 7 days before sampling	Time [h] above threshold 7 days before sampling
1	16.73225	14.7905	17.855	0
2	16.48972917	15.073	17.67	0
3	16.59283036	14.891	18.6165	0
4	16.6840625	14.7115	18.3865	0
5	16.43900893	14.442	17.9965	0
6	20.15209524	16.2195	22.751	89
7	21.06463393	19.271	22.8785	145
8	19.51587202	16.007	23.678	63
9	19.7430241	15.397	23.6715	67
10	19.67175	19.575	19.7685	0
11	18.89392857	18.797	18.9905	0
12	17.29922321	15.085	18.863	0
13	15.51952976	13.2645	17.386	0
14	15.60387202	14.276	16.8015	0
15	16.18684731	14.431	18.3945	0
16	16.23176488	14.93	18.906	0
17	20.99294643	17.8235	23.6485	116
18	17.6847619	14.7905	20.9805	18
19	21.59413095	18.249	24.333	148
20	18.87680357	16.127	21.7875	37
21	16.10402679	14.66	17.401	0
22	16.15078571	14.842	17.462	0
23	16.3927619	15.0395	18.051	0

DA Table B 12: Cooling Tower 1 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 10 days.

Sample #	Mean water temp. [°C] 10 days before sampling	Min. water temp. [°C] 10 days before sampling	Max. water temp. [°C] 10 days before sampling	Time [h] above threshold 10 days before sampling
1	17.31401316	16.354	18.5905	0
2	17.0670625	15.1625	18.3615	0
3	17.7445125	16.2125	19.2785	0
4	17.15265	13.2505	19.314	0
5	17.91875641	16.075	20.7165	12
6	18.0572875	15.84	21.1545	18
7	19.5126125	16.355	22.9325	78
8	18.6303625	16.0475	21.086	42
9	17.71035	16.329	19.9655	0
10	17.7615	16.2755	19.137	0
11	17.2886125	15.589	19.003	0
12	16.9350875	15.676	18.8515	0
13	17.4681875	16.031	19.1995	0
14	17.6486625	15.733	19.127	0
15	17.47105	15.426	20.245	6
16	16.9267125	14.646	18.8115	0
17	17.4851875	15.518	19.411	0
18	17.59465	15.955	19.386	0
19	17.926425	16.0925	19.04	0
20	19.39185	16.5405	22.8305	78
21	18.525775	16.4125	20.961	18
22	17.763525	16.1495	19.5705	0
23	17.5350125	16.2695	19.0475	0
24	17.4245125	15.992	19.1915	0
25	17.6332375	15.613	19.2745	0
26	17.5081125	15.6185	19.074	0
27	17.71939744	15.609	19.01	0
28	17.7318	16.5465	19.4345	0
29	17.7094375	15.616	19.215	0
30	19.2745875	16.727	23.46	66
31	18.77869231	16.3855	22.627	66
32	21.038175	18.4755	23.5415	204
33	18.0688125	15.652	19.9415	0
34	18.1415375	16.363	19.4615	0
35	17.8640375	16.528	19.289	0
36	17.57414103	15.6655	19.1615	0
37	17.4593	15.8255	18.728	0
38	17.70385	15.669	19.345	0
39	17.354025	15.673	19.5235	0
40	17.262775	15.8475	19.083	0
41	17.67286842	16.206	19.695	0
42	17.7103125	16.45	19.382	0
43	20.2905625	16.142	24.736	102
44	18.339025	16.32	22.5525	18
45	17.787025	14.7025	19.444	0
46	17.773825	16.4025	18.9965	0

Sample #	Mean water temp. [°C] 10 days before sampling	Min. water temp. [°C] 10 days before sampling	Max. water temp. [°C] 10 days before sampling	Time [h] above threshold 10 days before sampling
47	17.979875	16.604	19.933	0
48	17.415	16.101	19.1115	0
49	17.7911125	16.2705	19.678	0
50	17.5346125	16.162	18.9285	0
51	15.9171875	14.3015	17.948	0
52	16.3028625	15.0765	18.63	0
53	16.8176125	15.2775	18.6425	0
54	19.5500375	17.362	21.608	84
55	17.920525	15.8	22.948	24
56	19.3511125	15.744	22.1725	78
57	18.6629625	15.517	24.525	42
58	16.8811375	15.2195	18.4335	0
59	16.731725	15.2565	18.0525	0
60	16.6264125	15.2575	18.3795	0
61	16.67688953	14.6475	17.855	0
62	16.57836875	15.073	18.332	0
63	16.51633333	14.891	18.6165	0
64	16.61501674	14.7115	18.3865	0
65	16.50212292	14.442	17.9965	0
66	19.57923958	15.325	24.456	110
67	20.20699792	16.2195	22.751	143
68	20.82259583	18.945	22.8785	175
69	19.72202708	16.007	23.678	101
70	19.40734454	15.397	23.6715	81
71	19.71406224	19.575	19.853	0
72	18.93565833	18.797	19.074	0
73	17.39952304	15.085	19.7635	0
74	15.54410208	13.2645	17.386	0
75	15.49769583	14.276	16.8015	0
76	16.15586402	14.431	18.3945	0
77	16.31338333	14.93	19.037	0
78	21.03217083	17.8235	23.6485	178
79	17.4282375	14.7905	20.9805	18
80	21.5672125	18.249	24.333	214
81	18.48888958	15.533	21.7875	39
82	16.08271875	14.5295	19.2115	0
83	16.09707676	14.4145	17.462	0
84	16.26564375	14.973	18.051	0

DA Table B 13: Cooling Tower 1 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 10 days.

Sample #	Mean water temp. [°C] 10 days before sampling	Min. water temp. [°C] 10 days before sampling	Max. water temp. [°C] 10 days before sampling	Time [h] above threshold 10 days before sampling
1	16.67688953	14.6475	17.855	0
2	16.57836875	15.073	18.332	0
3	16.51633333	14.891	18.6165	0
4	16.61501674	14.7115	18.3865	0
5	16.50212292	14.442	17.9965	0
6	20.20699792	16.2195	22.751	143
7	20.82259583	18.945	22.8785	175
8	19.72202708	16.007	23.678	101
9	19.40734454	15.397	23.6715	81
10	19.71406224	19.575	19.853	0
11	18.93565833	18.797	19.074	0
12	17.39952304	15.085	19.7635	0
13	15.54410208	13.2645	17.386	0
14	15.49769583	14.276	16.8015	0
15	16.15586402	14.431	18.3945	0
16	16.31338333	14.93	19.037	0
17	21.03217083	17.8235	23.6485	178
18	17.4282375	14.7905	20.9805	18
19	21.5672125	18.249	24.333	214
20	18.48888958	15.533	21.7875	39
21	16.08271875	14.5295	19.2115	0
22	16.09707676	14.4145	17.462	0
23	16.26564375	14.973	18.051	0

DA Table B 14: Cooling Tower 2 – Microbiological concentrations from the Laboratory of Technical Hygiene, IHPH

Legionella Sample #	Sampling Date	Sampling Time	*Legionella* [cfu/100 mL]	Serology	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	HPC Sample #
1	10.01.2012	10:20:00	10	2 - 14			
2	31.01.2012	10:10:00	10	2 - 14			
3	06.03.2012	11:10:00	25	2 - 14			
4	03.04.2012	10:20:00	105	1			
5	08.05.2012	11:30:00	70	1			
6	05.06.2012	09:55:00	490	1			
7	10.07.2012	10:10:00	120	1			
8	07.08.2012	09:35:00	45	1			
9	04.09.2012	10:30:00	1150	1, 2 - 14			
10	01.10.2012	10:10:00	1000	1			
11	06.11.2012	10:20:00	640	1			
12	04.12.2012	10:40:00	2750	1			
13	08.01.2013	10:10:00	520	1			
14	04.02.2013	09:55:00	2000	1, 2 - 14			
15	05.03.2013	10:25:00	350	1, 2 - 14			
16	02.04.2013	09:40:00	150	1			
17	06.05.2013	09:40:00	650	1			
18	03.06.2013	12:05:00	335	1			
19	02.07.2013	09:55:00	185	1			
20	13.08.2013	09:45:00	10	1			
21	03.09.2013	09:55:00	2600	1			
22	24.09.2013	10:35:00	2800	1			
23	05.11.2013	12:45:00	130	2 - 14			
24	03.12.2013	13:50:00	50	1			
25	14.01.2014	10:45:00	<DL				
26	11.02.2014	11:40:00	35	1			
27	11.03.2014	12:10:00	50	1, 2 - 14			
28	08.04.2014	10:15:00	600	1, 2 - 14			
29	06.05.2014	11:10:00	1010	1, 2 - 14			
30	10.06.2014	12:00:00	6050	1			
31	08.07.2014	16:30:00	700	2 - 14			
32	05.08.2014	13:20:00	3500	2 - 14			
33	02.09.2014	12:20:00	660	1, 2 - 14			
34	07.10.2014	10:50:00	1700	1			
35	04.11.2014	13:50:00	300	1, 2 - 14			
36	02.12.2014	12:20:00	515	2 - 14			
37	13.01.2015	11:20:00	250	1			
38	10.02.2015	11:15:00	45	1			
39	10.03.2015	11:10:00	80	1, 2 - 14			
40	07.04.2015	10:25:00	90	1, 2 - 14			
41	05.05.2015	13:05:00	200	2 - 14			
42	02.06.2015	10:50:00	425	2 - 14			
43	07.07.2015	11:10:00	8900	2 - 14			
44	04.08.2015	09:50:00	750	2 - 14			
45	16.09.2015	10:00:00	1550	2 - 14			
46	06.10.2015	14:30:00	250	2 - 14			
47	17.11.2015	11:30:00	145	1			

Legionella Sample #	Sampling Date	Sampling Time	*Legionella* [cfu/100 mL]	Serology	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	HPC Sample #
48	01.12.2015	11:20:00	255	1, *L. sp.*			
49	12.01.2016	11:20:00	100	1			
50	02.02.2016	10:05:00	75	1			
51	07.03.2016	12:40:00	500	1, 2 - 14			
52	06.04.2016	09:45:00	70	2 - 14			
53	11.05.2016	09:25:00	205	2 - 14			
54	07.06.2016	10:23:00	1200	1, 2 - 14			
55	04.07.2016	09:21:00	1200	1, 2 - 14			
56	02.08.2016	17:25:00	515	2 - 14			
57	06.09.2016	09:50:00	280	1			
58	04.10.2016	10:42:00	290	2 - 14			
59	02.11.2016	10:43:00	475	2 - 14			
60	06.12.2016	09:58:00	550	2 - 14			
61	03.01.2017	10:50:00	200	1, 2 - 14	1080	3240	1
62	07.02.2017	09:37:00	355	2 - 14	5400	910	2
63	07.03.2017	09:42:00	150	2 - 14	40	66	3
64	04.04.2017	10:22:00	500	2 - 14	240	260	4
65	02.05.2017	09:55:00	465	1	540	300	5
66	06.06.2017	09:15:00	12900	2 - 14			
67	03.07.2017	09:58:00	250	2 - 14	70200	70200	6
68	01.08.2017	10:30:00	1000	1, 2 - 14	75600	6480	7
69	05.09.2017	12:08:00	1600	2 - 14	20400	30000	8
70	04.10.2017	10:36:00	2200	2 - 14	1080	960	9
71	07.11.2017	10:50:00	500	2 - 14	144	260	10
72	05.12.2017	10:24:00	200	1, 2 - 14	70	240	11
73	09.01.2018	10:45:00	20	2 - 14	30	110	12
74	06.02.2018	10:47:00	40	2 - 14	50	100	13
75	06.03.2018	11:55:00	<DL		100	330	14
76	10.04.2018	10:03:00	13000	2 - 14	820	530	15
	23.04.2018				570	1800	16
77	08.05.2018	09:15:00	35	2 - 14	1800	1800	17
78	05.06.2018	10:31:00	1300	2 - 14	20	540	18
79	03.07.2018	10:38:00	1300	2 - 14	1580	1940	19
80	07.08.2018	10:15:00	2200	2 - 14	950	280	20
81	04.09.2018	08:00:00	2700	2 - 14	10	10	21
82	01.10.2018	11:30:00	550	2 - 14	270	270	22
83	05.11.2018	09:24:00	180	2 - 14	100	60	23
84	03.12.2018	09:06:00	220	2 - 14	1180	980	24
	07.01.2019	09:26:00	18		120	130	25
	04.02.2019	09:47:00	55				
	05.03.2019	09:01:00	35		100	30	26
	01.04.2019	10:35:00	5		170	160	27
	06.05.2019	10:05:00	10		780	860	28
	03.06.2019	10:09:00	<DL		220	330	29
	01.07.2019	09:33:00	<DL		1	20	30
	05.08.2019	09:40:00	<DL		6600	13500	31
	02.09.2019	10:16:00	20		160	20	32
	07.10.2019	10:05:00	<DL		280	1	33

Legionella Sample #	Sampling Date	Sampling Time	*Legionella* [cfu/100 mL]	Serology	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	HPC Sample #
	04.11.2019	12:32:00	5		260	1090	34
	02.12.2019	09:58:00	15		170	70	35
	06.01.2020	10:43:00	<DL		780	500	
	03.02.2020	10:49:00	<DL		410	190	
	02.03.2020	09:45:00	<DL		50	130	
	06.04.2020	09:42:00	<DL		580	1800	
	04.05.2020	10:30:00	5		1020	1000	
	02.06.2020	10:09:00	15		720	310	
	06.07.2020	10:18:00	<DL		730	710	
	03.08.2020	09:51:00	5		670	640	
	07.09.2020	10:45:00	<DL		140	120	

DA Table B 15: Cooling Tower 2 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 350 mV, observation period: 5 days.

Sample #	Biocide dosages 5 days before sampling	Biocide dosages 5 days after sampling	Biocide dosages 5 days before and after sampling	Ratio of dosages 5 days after and before sampling	Mean mV 5 days before sampling	Time [h] below threshold 5 days before sampling
1	14	23	37	1.642857143	374.7279167	18
2	26	33	59	1.269230769	397.99475	0
3	24	28	52	1.166666667	401.545	1
4	20	26	46	1.3	397.4410833	0
5	23	21	44	0.913043478	397.4878333	0
6	35	24	59	0.685714286	397.1981667	1
7	26	21	47	0.807692308	396.1256667	0
8	37	29	66	0.783783784	396.21	1
9	17	9	26	0.529411765	398.1029167	0
10	9	20	29	2.222222222	397.4099167	0
11	6	10	16	1.666666667	398.1854167	0
12	15	15	30	1	398.4310833	0
13	20	18	38	0.9	402.7183215	1
14	26	27	53	1.038461538	396.3665782	3
15	24	11	35	0.458333333	395.0120946	8
16	27	25	52	0.925925926	398.1388398	1
17	22	24	46	1.090909091	396.1163635	6
18	14	19	33	1.357142857	399.0405398	0
19	27	22	49	0.814814815	399.6354673	1
20	23	20	43	0.869565217	527.5242569	0
21	32	35	67	1.09375	397.9591385	0
22	25	27	52	1.08	401.4248975	5
23	19	21	40	1.105263158	396.86701	0
24	29	28	57	0.965517241	398.3743131	0
25	31	34	65	1.096774194	398.9042384	0
26	35	25	60	0.714285714	400.1331178	3
27	27	32	59	1.185185185	398.117676	0
28	33	28	61	0.848484848	397.6087611	0
29	31	29	60	0.935483871	396.3233012	3
30	27	24	51	0.888888889	396.6169795	0
31	25	19	44	0.76	376.8811707	46
32	27	12	39	0.444444444	437.4391912	0
33	21	22	43	1.047619048	443.6331627	1
34	38	27	65	0.710526316	451.2235792	2
35	22	27	49	1.227272727	462.6980919	0
36	36	35	71	0.972222222	447.6854907	0
37	27	26	53	0.962962963	431.9435036	2
38	15	14	29	0.933333333	397.4496173	2
39	32	25	57	0.78125	458.7235596	0
40	21	22	43	1.047619048	456.8625585	0
41	16	23	39	1.4375	451.6940025	1
42	27	34	61	1.259259259	457.2300316	0
43	38	27	65	0.710526316	455.8309858	0
44	22	24	46	1.090909091	460.2189697	0
45	51	47	98	0.921568627	455.9622957	0
46	31	28	59	0.903225806	455.9026133	0
47	24	29	53	1.208333333	457.5747119	0

Sample #	Biocide dosages 5 days before sampling	Biocide dosages 5 days after sampling	Biocide dosages 5 days before and after sampling	Ratio of dosages 5 days after and before sampling	Mean mV 5 days before sampling	Time [h] below threshold 5 days before sampling
48	28	29	57	1.035714286	465.4397659	0
49	37	40	77	1.081081081	469.523644	0
50	27	25	52	0.925925926	459.1564578	0
51	24	40	64	1.666666667	462.9639898	0
52	35	38	73	1.085714286	457.7441925	0
53	37	29	66	0.783783784	456.7498456	1
54	29	9	38	0.310344828	452.6581718	1
55	21	14	35	0.666666667	381.6454559	57
56	30	36	66	1.2	446.5127068	8
57	13	8	21	0.615384615	595.0467219	0
58	15	13	28	0.866666667	466.6882584	0
59	28	25	53	0.892857143	452.0986664	1
60	20	19	39	0.95	446.750902	16
61	26	20	46	0.769230769	466.6090701	0
62	20	2	22	0.1	438.8487823	14
63	16	27	43	1.6875	371.4357521	63
64	33	31	64	0.939393939	450.9206713	4
65	25	37	62	1.48	430.7772926	25
66	10	38	48	3.8	345.5288432	96
67	40	44	84	1.1	456.6336988	0
68	40	38	78	0.95	458.1282321	0
69	16	18	34	1.125	405.7842868	42
70	23	9	32	0.391304348	432.6233601	20
71	0	0	0	0	414.6124034	0
72	0	10	10	10	415.1344376	0
73	24	27	51	1.125	469.5014613	0
74	36	31	67	0.861111111	464.8130506	0
75	32	30	62	0.9375	452.6745453	0
76	42	34	76	0.80952381	457.7647199	0
77	28	26	54	0.928571429	476.369886	0
78	26	22	48	0.846153846	475.0196922	0
79	35	36	71	1.028571429	494.9542727	0
80	17	15	32	0.882352941	441.8755191	2
81	16	14	30	0.875	473.3520723	1
82	22	21	43	0.954545455	489.4169858	0
83	24	12	36	0.5	490.7830869	2
84	15	18	33	1.2	338.2197756	83

DA Table B 16: Cooling Tower 2 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 350 mV, observation period: 5 days.

Sample #	Biocide dosages 5 days before sampling	Biocide dosages 5 days after sampling	Biocide dosages 5 days before and after sampling	Ratio of dosages 5 days after and before sampling	Mean mV 5 days before sampling	Time [h] below threshold 5 days before sampling
1	25	20	45	0.8	466.9428581	0
2	20	2	22	0.1	438.8487823	14
3	16	27	43	1.6875	371.4357521	63
4	33	31	64	0.939393939	450.9206713	4
5	25	37	62	1.48	430.7772926	25
6	40	44	84	1.1	456.6336988	0
7	40	38	78	0.95	458.1282321	0
8	16	18	34	1.125	405.7842868	42
9	23	9	32	0.391304348	432.6233601	20
10	0	0	0	0	414.6124034	0
11	0	10	10	10	415.1344376	0
12	24	27	51	1.125	469.5014613	0
13	36	31	67	0.861111111	464.8130506	0
14	32	30	62	0.9375	452.6745453	0
15	42	34	76	0.80952381	457.7647199	0
16	17	22	39	1.294117647	440.1373402	20
17	28	26	54	0.928571429	476.369886	0
18	26	22	48	0.846153846	475.0196922	0
19	35	36	71	1.028571429	494.9542727	0
20	17	15	32	0.882352941	441.8755191	2
21	16	14	30	0.875	473.3520723	1
22	22	21	43	0.954545455	489.4169858	0
23	24	12	36	0.5	490.7830869	2
24	15	18	33	1.2	338.2197756	83
25	20	14	34	0.7	394.7486392	0
26	28	29	57	1.035714286	337.9809929	91
27	18	28	46	1.555555556	332.9804675	91
28	21	24	45	1.142857143	310.5953354	120
29	31	26	57	0.838709677	312.2038678	120
30	27	0	27	0	301.2255361	120
31	20	0	20	0	329.5582703	113
32	20	0	20	0	310.2447001	117
33	22	0	22	0	363.2610507	4
34	29	0	29	0	355.4643542	16
35	24	0	24	0	344.1640176	94

DA Table B 17: Cooling Tower 2 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 350 mV, observation period: 7 days.

Sample #	Biocide dosages 7 days before sampling	Biocide dosages 7 days after sampling	Biocide dosages 7 days before and after sampling	Ratio of dosages 7 days after and before sampling	Mean mV 7 days before sampling	Time [h] below threshold 7 days before sampling
1	17	33	50	1.941176471	362.341131	52
2	36	41	77	1.138888889	397.8999405	0
3	35	39	74	1.114285714	400.4895238	1
4	28	30	58	1.071428571	397.6550595	0
5	27	26	53	0.962962963	394.3452381	6
6	44	31	75	0.704545455	397.2039286	1
7	39	34	73	0.871794872	396.8425	0
8	52	37	89	0.711538462	395.8040476	1
9	29	16	45	0.551724138	397.8875	0
10	14	28	42	2	397.5064286	0
11	9	11	20	1.222222222	398.5959524	0
12	24	25	49	1.041666667	398.3890476	0
13	25	22	47	0.88	401.7722569	1
14	36	33	69	0.916666667	396.7659358	3
15	34	18	52	0.529411765	396.7189465	8
16	36	35	71	0.972222222	399.0552522	1
17	31	34	65	1.096774194	396.7203934	6
18	22	29	51	1.318181818	395.760966	1
19	37	29	66	0.783783784	398.980276	1
20	27	33	60	1.222222222	513.1896099	0
21	43	55	98	1.279069767	398.4889374	0
22	29	31	60	1.068965517	399.5750571	7
23	26	37	63	1.423076923	396.7488614	0
24	41	38	79	0.926829268	398.3109382	2
25	40	47	87	1.175	393.3408897	8
26	44	30	74	0.681818182	391.501087	9
27	37	47	84	1.27027027	398.2407441	0
28	41	33	74	0.804878049	397.6150449	0
29	39	36	75	0.923076923	396.7026907	3
30	33	35	68	1.060606061	397.1821814	0
31	32	26	58	0.8125	386.2018558	53
32	38	19	57	0.5	441.9227475	0
33	26	32	58	1.230769231	420.3065293	7
34	52	39	91	0.75	452.9279233	2
35	32	40	72	1.25	460.9628447	0
36	55	54	109	0.981818182	450.2531586	0
37	33	37	70	1.121212121	427.6528404	2
38	26	26	52	1	414.1281266	2
39	41	36	77	0.87804878	460.6203851	0
40	30	33	63	1.1	457.5904441	0
41	24	37	61	1.541666667	453.467976	1
42	39	45	84	1.153846154	454.4123895	0
43	51	37	88	0.725490196	455.170552	0
44	32	39	71	1.21875	462.4075219	0
45	70	67	137	0.957142857	455.7835787	0
46	49	38	87	0.775510204	454.2555136	1
47	35	45	80	1.285714286	457.8199274	0

Sample #	Biocide dosages 7 days before sampling	Biocide dosages 7 days after sampling	Biocide dosages 7 days before and after sampling	Ratio of dosages 7 days after and before sampling	Mean mV 7 days before sampling	Time [h] below threshold 7 days before sampling
48	44	46	90	1.045454545	463.4053263	0
49	54	51	105	0.944444444	469.1556978	0
50	42	43	85	1.023809524	459.6520384	0
51	30	52	82	1.733333333	462.915356	0
52	49	53	102	1.081632653	457.8808719	0
53	48	40	88	0.833333333	457.3270444	1
54	40	10	50	0.25	447.8244952	6
55	33	25	58	0.757575758	403.6522758	57
56	43	49	92	1.139534884	450.2900805	8
57	20	13	33	0.65	577.4508147	8
58	22	26	48	1.181818182	464.686561	0
59	44	42	86	0.954545455	454.1560171	1
60	25	36	61	1.44	452.5399421	16
61	35	36	71	1.028571429	468.1606929	0
62	30	4	34	0.133333333	444.4394399	14
63	29	38	67	1.310344828	396.3163521	63
64	45	49	94	1.088888889	451.84021	4
65	36	58	94	1.611111111	432.9636617	29
66	20	53	73	2.65	369.7078053	105
67	52	60	112	1.153846154	454.9565362	1
68	51	50	101	0.980392157	457.8685931	0
69	27	24	51	0.888888889	420.6855294	42
70	31	11	42	0.35483871	437.2135161	21
71	0	0	0	0	414.5937594	0
72	0	28	28	28	415.1157935	0
73	33	27	60	0.818181818	473.059775	0
74	52	40	92	0.769230769	465.2120389	0
75	42	43	85	1.023809524	447.1992093	9
76	62	49	111	0.790322581	457.552831	0
77	43	35	78	0.813953488	476.3406054	0
78	36	33	69	0.916666667	475.1677922	0
79	51	56	107	1.098039216	495.0448325	0
80	20	25	45	1.25	437.3697933	2
81	20	26	46	1.3	466.8271753	1
82	28	28	56	1	490.635968	0
83	32	19	51	0.59375	463.5572154	24
84	19	23	42	1.210526316	336.8362071	118

DA Table B 18: Cooling Tower 2 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 350 mV, observation period: 7 days.

Sample #	Biocide dosages 7 days before sampling	Biocide dosages 7 days after sampling	Biocide dosages 7 days before and after sampling	Ratio of dosages 7 days after and before sampling	Mean mV 7 days before sampling	Time [h] below threshold 7 days before sampling
1	35	35	70	1	468.859599	0
2	30	4	34	0.133333333	444.4394399	14
3	29	38	67	1.310344828	396.3163521	63
4	45	49	94	1.088888889	451.84021	4
5	36	58	94	1.611111111	432.9636617	29
6	52	60	112	1.153846154	454.9565362	1
7	51	50	101	0.980392157	457.8685931	0
8	27	24	51	0.888888889	420.6855294	42
9	31	11	42	0.35483871	437.2135161	21
10	0	0	0	0	414.5937594	0
11	0	28	28	28	415.1157935	0
12	33	27	60	0.818181818	473.059775	0
13	52	40	92	0.769230769	465.2120389	0
14	42	43	85	1.023809524	447.1992093	9
15	62	49	111	0.790322581	457.552831	0
16	30	35	65	1.166666667	445.0581858	20
17	43	35	78	0.813953488	476.3406054	0
18	36	33	69	0.916666667	475.1677922	0
19	51	56	107	1.098039216	495.0448325	0
20	20	25	45	1.25	437.3697933	2
21	20	26	46	1.3	466.8271753	1
22	28	28	56	1	490.635968	0
23	32	19	51	0.59375	463.5572154	24
24	19	23	42	1.210526316	336.8362071	118
25	27	25	52	0.925925926	396.4300041	0
26	37	43	80	1.162162162	333.2524707	139
27	25	38	63	1.52	321.8148541	139
28	28	33	61	1.178571429	315.4293631	168
29	43	30	73	0.697674419	312.9661613	168
30	36	0	36	0	298.1868488	168
31	30	0	30	0	323.8481338	161
32	31	0	31	0	311.5769121	165
33	34	0	34	0	362.1995759	6
34	45	0	45	0	356.6006165	21
35	33	0	33	0	345.0102567	133

DA Table B 19: Cooling Tower 2 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 350 mV, observation period: 10 days.

Sample #	Biocide dosages 10 days before sampling	Biocide dosages 10 days after sampling	Biocide dosages 10 days before and after sampling	Ratio of dosages 10 days after and before sampling	Mean mV 10 days before sampling	Time [h] below threshold 10 days before sampling
1	19	46	65	2.421052632	355.4350442	98
2	55	53	108	0.963636364	396.6683333	6
3	46	57	103	1.239130435	400.2415	1
4	35	38	73	1.085714286	397.6887866	0
5	48	37	85	0.770833333	384.1008333	23
6	54	44	98	0.814814815	397.745875	1
7	58	55	113	0.948275862	397.2462917	0
8	74	53	127	0.716216216	395.2960833	2
9	45	28	73	0.622222222	397.8137083	0
10	23	42	65	1.826086957	397.5144167	0
11	14	11	25	0.785714286	398.4958091	0
12	37	40	77	1.081081081	398.2899167	0
13	34	32	66	0.941176471	400.962166	1
14	50	49	99	0.98	397.5381042	3
15	45	30	75	0.666666667	397.7939798	8
16	44	44	88	1	406.5209955	2
17	41	51	92	1.243902439	399.5335856	6
18	30	43	73	1.433333333	398.4726227	1
19	50	40	90	0.8	399.1288162	2
20	36	57	93	1.583333333	520.73451	0
21	63	78	141	1.238095238	398.7012438	2
22	38	34	72	0.894736842	400.3648253	10
23	35	59	94	1.685714286	396.7721579	0
24	64	53	117	0.828125	397.9134664	4
25	52	62	114	1.192307692	393.5550195	8
26	48	45	93	0.9375	397.0278819	10
27	55	69	124	1.254545455	398.535424	0
28	53	40	93	0.754716981	397.7728577	0
29	46	45	91	0.97826087	399.1040103	3
30	42	55	97	1.30952381	397.1421662	1
31	49	32	81	0.653061224	385.7530801	85
32	55	32	87	0.581818182	448.2786672	0
33	37	38	75	1.027027027	421.9359769	7
34	69	50	119	0.724637681	449.7656803	8
35	51	62	113	1.215686275	460.2522187	0
36	85	80	165	0.941176471	450.9091723	0
37	44	50	94	1.136363636	424.7727515	4
38	36	42	78	1.166666667	434.5526183	3
39	62	41	103	0.661290323	458.8553956	0
40	43	44	87	1.023255814	456.7512806	0
41	38	53	91	1.394736842	453.6796062	2
42	58	58	116	1	455.6376284	0
43	76	52	128	0.684210526	455.6613022	0
44	50	57	107	1.14	461.9161044	0
45	100	91	191	0.91	455.8393246	0
46	74	49	123	0.662162162	451.3598241	8

Sample #	Biocide dosages 10 days before sampling	Biocide dosages 10 days after sampling	Biocide dosages 10 days before and after sampling	Ratio of dosages 10 days after and before sampling	Mean mV 10 days before sampling	Time [h] below threshold 10 days before sampling
47	49	68	117	1.387755102	459.0858032	0
48	67	66	133	0.985074627	461.545268	0
49	82	74	156	0.902439024	468.3783099	0
50	66	65	131	0.984848485	461.8718519	0
51	41	69	110	1.682926829	464.2698799	0
52	72	72	144	1	457.670547	0
53	69	55	124	0.797101449	457.3214252	1
54	52	19	71	0.365384615	445.3916866	6
55	53	43	96	0.811320755	419.9821739	57
56	67	63	130	0.940298507	453.0141588	8
57	24	20	44	0.833333333	502.1021454	80
58	33	42	75	1.272727273	464.0370328	0
59	65	59	124	0.907692308	457.6157458	1
60	32	60	92	1.875	458.3526564	16
61	50	57	107	1.14	469.2814219	0
62	50	6	56	0.12	447.9520645	14
63	47	53	100	1.127659574	412.9237446	64
64	65	70	135	1.076923077	453.554208	5
65	55	85	140	1.545454545	440.8251686	29
66	37	73	110	1.972972973	388.9384547	117
67	74	81	155	1.094594595	456.5629631	1
68	69	66	135	0.956521739	458.1415994	0
69	47	39	86	0.829787234	431.8842496	42
70	33	17	50	0.515151515	417.483683	65
71	0	0	0	0	414.5654047	0
72	0	37	37	37	415.0878273	0
73	49	44	93	0.897959184	471.5217799	0
74	78	55	133	0.705128205	464.0914528	1
75	50	61	111	1.22	434.0535365	25
76	91	62	153	0.681318681	457.8039191	0
77	63	48	111	0.761904762	476.9923773	0
78	55	48	103	0.872727273	475.4065584	0
79	79	78	157	0.987341772	495.7312262	0
80	34	33	67	0.970588235	449.0737011	2
81	31	35	66	1.129032258	428.1913737	42
82	40	34	74	0.85	492.1492087	0
83	48	26	74	0.541666667	473.382861	24
84	33	37	70	1.121212121	384.653	118

DA Table B 20: Cooling Tower 2 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 350 mV, observation period: 10 days.

Sample #	Biocide dosages 10 days before sampling	Biocide dosages 10 days after sampling	Biocide dosages 10 days before and after sampling	Ratio of dosages 10 days after and before sampling	Mean mV 10 days before sampling	Time [h] below threshold 10 days before sampling
1	50	57	107	1.14	469.5048981	0
2	50	6	56	0.12	447.9520645	14
3	47	53	100	1.127659574	412.9237446	64
4	65	70	135	1.076923077	453.554208	5
5	55	85	140	1.545454545	440.8251686	29
6	74	81	155	1.094594595	456.5629631	1
7	69	66	135	0.956521739	458.1415994	0
8	47	39	86	0.829787234	431.8842496	42
9	33	17	50	0.515151515	417.483683	65
10	0	0	0	0	414.5654047	0
11	0	37	37	37	415.0878273	0
12	49	44	93	0.897959184	471.5217799	0
13	78	55	133	0.705128205	464.0914528	1
14	50	61	111	1.22	434.0535365	25
15	91	62	153	0.681318681	457.8039191	0
16	53	58	111	1.094339623	448.8869963	20
17	63	48	111	0.761904762	476.9923773	0
18	55	48	103	0.872727273	475.4065584	0
19	79	78	157	0.987341772	495.7312262	0
20	34	33	67	0.970588235	449.0737011	2
21	31	35	66	1.129032258	428.1913737	42
22	40	34	74	0.85	492.1492087	0
23	48	26	74	0.541666667	473.382861	24
24	33	37	70	1.121212121	384.653	118
25	38	33	71	0.868421053	403.6243474	0
26	52	65	117	1.25	333.7085698	203
27	36	51	87	1.416666667	319.7185061	211
28	38	45	83	1.184210526	324.7110238	224
29	56	30	86	0.535714286	319.1105428	215
30	48	0	48	0	305.3042856	240
31	44	0	44	0	318.519305	233
32	43	0	43	0	314.5290732	237
33	49	0	49	0	358.7834338	38
34	60	0	60	0	359.1629503	22
35	46	0	46	0	351.6190411	141

DA Table B 21: Cooling Tower 2 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 5 days.

Sample #	Mean water temp. [°C] 5 days before sampling	Min. water temp. [°C] 5 days before sampling	Max. water temp. [°C] 5 days before sampling	Time [h] above threshold 5 days before sampling
1	16.69701667	14.555	19.53366667	0
2	17.51725	15.034	22.071	12
3	16.6786	13.70166667	18.819	0
4	17.72765	15.034	20.56266667	6
5	14.91698333	12.13433333	17.25833333	0
6	22.29485	20.307	25.75775	120
7	21.5920625	19.6455	24.35225	108
8	19.5591125	17.36625	21.00525	36
9	20.982525	17.7855	24.863	78
10	22.8004125	20.18925	24.306	120
11	26.99083333	25.14166667	28.74133333	120
12	23.37001667	22.12666667	24.33933333	120
13	23.49688333	21.81333333	25.16366667	120
14	24.18161404	21.909	28.629	120
15	21.69616667	20.447	25.04633333	120
16	18.43408333	16.345	20.838	12
17	19.7736625	15.205	23.422	60
18	20.7178375	18.501	23.21375	84
19	22.9449375	18.99275	25.29375	114
20	20.060675	17.67325	21.22125	78
21	24.2070375	18.79	27.19975	114
22	21.518225	17.47075	27.82125	60
23	18.2622125	16.535	19.89675	0
24	19.7401	17.391	21.98	54
25	14.5279375	12.537	17.6525	0
26	18.836	17.4115	21.2165	18
27	20.506675	17.52075	23.37625	78
28	21.1969625	18.23175	23.705	84
29	19.5232875	16.31475	22.251	48
30	25.27535526	20.698	28.61925	114
31	24.5451	22.29525	26.55125	120
32	23.8461875	22.09075	24.733	120
33	20.1499625	17.21525	23.6565	60
34	22.4484375	18.2535	24.4635	114
35	21.41405	18.02366667	27.019	72
36	15.90018333	12.95966667	19.797	0
37	16.31923333	12.87333333	20.25433333	6
38	13.933075	10.3375	18.3645	0
39	16.7565625	14.58175	18.665	0
40	19.79588333	17.752	22.32133333	42
41	20.75259259	16.50133333	25.91766667	66
42	22.53211667	20.4	24.76266667	120
43	29.0715	24.472	32.80266667	120
44	22.20113333	18.18966667	27.527	96
45	29.782375	27.014	32.6785	120
46	18.499925	15.248	20.90125	18

Sample #	Mean water temp. [°C] 5 days before sampling	Min. water temp. [°C] 5 days before sampling	Max. water temp. [°C] 5 days before sampling	Time [h] above threshold 5 days before sampling
47	21.25565	18.672	23.29366667	102
48	18.45493333	16.38	20.555	12
49	15.8036125	13.73625	18.43425	0
50	22.47183333	19.83133333	25.134	114
51	18.16605	16.6375	19.73275	0
52	19.522375	17.4525	21.20575	48
53	22.2647625	18.98725	26.25475	102
54	25.1842875	22.101	28.615	120
55	22.6251875	19.9005	26.97225	108
56	25.8389	23.05825	29.04925	120
57	21.8474625	19.3135	24.15175	108
58	18.9276875	16.82075	21.919	24
59	21.197275	17.75975	24.189	101
60	17.0200125	13.22225	21.1685	30
61	16.85866667	13.66775	19.40875	0
62	19.35469792	16.63575	22.364	47
63	17.42699792	14.209	21.98475	14
64	20.70161667	15.4565	25.06025	75
65	19.86211667	16.63475	21.80325	54
66	25.31737917	20.5255	29.863	120
67	23.81328125	19.74325	27.491	118
68	24.21348542	22.4505	26.52325	120
69	21.49273125	18.2225	26.45875	96
70	23.32489336	17.70175	27.98625	109
71	23.44605417	23.01025	23.88175	120
72	18.52396667	18.088	18.95975	0
73	13.99682917	11.59525	17.86925	0
74	13.84502292	12.126	15.64725	0
75	14.93885625	11.9545	18.47525	0
76	16.90808333	14.34575	21.9405	9
77	17.24956875	15.384	19.88575	0
78	22.16817917	19.767	23.934	117
79	19.91349375	17.77575	21.57675	64
80	18.59511667	14.8225	22.4545	33
81	23.12531092	17.62525	27.16175	108
82	18.15889375	12.6965	23.3	27
83	20.78105	17.4995	23.5315	92
84	21.61529583	18.3595	24.141	96

DA Table B 22: Cooling Tower 2 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 5 days.

Sample #	Mean water temp. [°C] 5 days before sampling	Min. water temp. [°C] 5 days before sampling	Max. water temp. [°C] 5 days before sampling	Time [h] above threshold [°C] 5 days before sampling
1	16.64803017	13.66775	19.40875	0
2	19.35469792	16.63575	22.364	47
3	17.42699792	14.209	21.98475	14
4	20.70161667	15.4565	25.06025	75
5	19.86211667	16.63475	21.80325	54
6	23.81328125	19.74325	27.491	118
7	24.21348542	22.4505	26.52325	120
8	21.49273125	18.2225	26.45875	96
9	23.32489336	17.70175	27.98625	109
10	23.44605417	23.01025	23.88175	120
11	18.52396667	18.088	18.95975	0
12	13.99682917	11.59525	17.86925	0
13	13.84502292	12.126	15.64725	0
14	14.93885625	11.9545	18.47525	0
15	16.90808333	14.34575	21.9405	9
16	19.77677917	16.342	22.03975	58
17	17.24956875	15.384	19.88575	0
18	22.16817917	19.767	23.934	117
19	19.91349375	17.77575	21.57675	64
20	18.59511667	14.8225	22.4545	33
21	23.12531092	17.62525	27.16175	108
22	18.15889375	12.6965	23.3	27
23	20.78105	17.4995	23.5315	92
24	21.61529583	18.3595	24.141	96

DA Table B 23: Cooling Tower 2 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 7 days.

Sample #	Mean water temp. [°C] 7 days before sampling	Min. water temp. [°C] 7 days before sampling	Max. water temp. [°C] 7 days before sampling	Time [h] above threshold 7 days before sampling
1	16.87554762	14.555	19.63466667	0
2	17.27113095	15.034	22.071	12
3	18.15590476	13.70166667	22.961	42
4	17.4480119	14.62533333	20.56266667	6
5	16.07771429	12.13433333	21.416	12
6	22.69978571	20.307	25.75775	168
7	21.71228571	19.6455	24.35225	156
8	19.74176786	17.36625	22.22875	60
9	21.73894643	17.7855	25.83975	126
10	23.12266964	20.18925	25.036	168
11	26.92079762	25.14166667	28.74133333	168
12	24.11311905	22.12666667	27.40333333	168
13	23.22458333	21.19166667	25.16366667	168
14	24.79462963	21.909	28.629	168
15	20.754	14.41333333	25.04633333	138
16	18.38207143	16.345	20.838	18
17	19.63591964	15.205	23.422	78
18	20.98891071	18.501	23.21375	126
19	22.86391071	18.99275	25.29375	162
20	20.62979464	17.67325	25.0495	120
21	23.23177679	18.79	27.19975	150
22	20.17145536	15.68575	27.82125	60
23	17.9450625	16.535	19.89675	0
24	19.51275	17.391	21.98	66
25	14.87357143	12.537	17.6525	0
26	18.72812037	16.2735	21.2165	18
27	19.66561607	15.07425	23.37625	78
28	21.3138125	18.23175	23.705	126
29	20.22596429	16.31475	23.35175	96
30	24.49936111	20.25925	28.61925	162
31	23.88340179	20.5195	26.55125	168
32	23.90546429	22.09075	26.65525	168
33	20.29997321	17.21525	23.6565	90
34	22.40160714	18.2535	24.4635	162
35	21.01682143	18.02366667	27.019	96
36	16.76766964	12.95966667	22.572	18
37	15.7335119	12.87333333	20.25433333	6
38	13.69985714	9.098	18.3645	0
39	16.1808125	13.6945	18.665	0
40	19.80578571	17.752	22.426	60
41	20.13338462	15.51566667	25.91766667	72
42	22.35644048	20.295	24.76266667	168
43	28.44188095	23.81666667	32.80266667	168
44	22.02538095	18.18966667	27.527	144
45	28.66322619	24.8135	32.6785	168
46	19.49039881	15.248	24.40166667	66

Sample #	Mean water temp. [°C] 7 days before sampling	Min. water temp. [°C] 7 days before sampling	Max. water temp. [°C] 7 days before sampling	Time [h] above threshold 7 days before sampling
47	21.351	18.672	23.29366667	150
48	18.41379167	16.38	20.555	12
49	16.2245625	13.73625	19.9505	0
50	22.18454464	18.29825	25.134	144
51	18.37035714	16.6375	20.20725	6
52	19.23436607	17.26125	21.20575	54
53	21.05873214	17.23375	26.25475	102
54	24.8305625	22.101	28.615	168
55	22.45413393	19.9005	26.97225	156
56	25.53911607	22.8085	29.04925	168
57	21.26654464	17.37125	24.15175	126
58	19.52685714	16.82075	23.34225	60
59	21.30301786	17.75975	24.189	137
60	16.06108929	8.2215	21.1685	30
61	16.72526014	13.66775	19.40875	0
62	19.27584524	16.499	23.92475	58
63	16.94031845	12.01175	21.98475	14
64	21.27623363	15.4565	25.06025	123
65	19.65411012	16.63475	21.80325	70
66	25.40525	20.5255	29.863	168
67	23.76834226	19.74325	27.491	166
68	24.60656548	22.4505	26.6025	168
69	23.22213839	18.2225	30.74325	144
70	23.75913956	17.70175	27.98625	157
71	23.62184673	23.01025	24.23325	168
72	18.69975	18.088	19.31125	0
73	14.28644643	11.59525	17.86925	0
74	14.28062798	12.126	18.078	0
75	14.69806101	11.79025	18.47525	0
76	17.30690327	14.34575	21.9405	9
77	17.07549851	13.66625	19.88575	0
78	22.42374107	19.767	24.74	165
79	19.6735372	17.145	21.57675	75
80	18.91183333	14.8225	22.4545	48
81	22.74626347	17.558	27.627	139
82	17.80291518	12.6965	23.3	27
83	20.39865774	17.0515	23.5315	109
84	21.27479762	18.3595	24.141	125

DA Table B 24: Cooling Tower 2 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 7 days.

Sample #	Mean water temp. [°C] 7 days before sampling	Min. water temp. [°C] 7 days before sampling	Max. water temp. [°C] 7 days before sampling	Time [h] above threshold 7 days before sampling
1	16.55405303	13.66775	19.40875	0
2	19.27584524	16.499	23.92475	58
3	16.94031845	12.01175	21.98475	14
4	21.27623363	15.4565	25.06025	123
5	19.65411012	16.63475	21.80325	70
6	23.76834226	19.74325	27.491	166
7	24.60656548	22.4505	26.6025	168
8	23.22213839	18.2225	30.74325	144
9	23.75913956	17.70175	27.98625	157
10	23.62184673	23.01025	24.23325	168
11	18.69975	18.088	19.31125	0
12	14.28644643	11.59525	17.86925	0
13	14.28062798	12.126	18.078	0
14	14.69806101	11.79025	18.47525	0
15	17.30690327	14.34575	21.9405	9
16	19.37498512	15.3795	22.03975	58
17	17.07549851	13.66625	19.88575	0
18	22.42374107	19.767	24.74	165
19	19.6735372	17.145	21.57675	75
20	18.91183333	14.8225	22.4545	48
21	22.74626347	17.558	27.627	139
22	17.80291518	12.6965	23.3	27
23	20.39865774	17.0515	23.5315	109
24	21.27479762	18.3595	24.141	125

DA Table B 25: Cooling Tower 2 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 10 days.

Sample #	Mean water temp. [°C] 10 days before sampling	Min. water temp. [°C] 10 days before sampling	Max. water temp. [°C] 10 days before sampling	Time [h] above threshold 10 days before sampling
1	17.91355263	14.555	23.25333333	30
2	17.53448333	15.034	22.071	18
3	18.48540833	13.70166667	22.961	66
4	18.72985833	14.62533333	28.32833333	48
5	16.85368333	12.13433333	21.416	24
6	22.70405	20.307	25.75775	240
7	21.33885625	17.46925	24.35225	204
8	19.60569375	17.262	22.4275	84
9	21.9859375	17.7855	25.83975	198
10	22.26309375	17.61525	25.036	210
11	25.91568333	20.451	28.74133333	239
12	25.11561667	22.12666667	29.04466667	240
13	24.10636667	21.19166667	28.33933333	240
14	24.55400855	21.909	28.629	240
15	18.742775	10.572	25.04633333	138
16	18.11149167	14.829	21.035	24
17	19.80359375	15.205	23.662	114
18	20.32029375	16.49525	23.21375	138
19	22.7222125	18.99275	25.29375	234
20	20.78948125	17.67325	25.063	168
21	23.614	18.79	29.817	210
22	20.25521875	14.869	27.82125	96
23	18.7386375	16.535	23.62125	47
24	18.83814375	15.9685	21.98	66
25	15.51476875	12.537	19.79625	0
26	18.61537821	15.7595	21.2165	24
27	19.03915	15.07425	23.37625	78
28	21.24716875	18.23175	23.705	186
29	20.440775	16.31475	23.35175	162
30	23.43807051	18.265	28.61925	222
31	23.5335875	20.5195	26.55125	240
32	24.3015	22.09075	26.65525	240
33	19.96810625	16.73925	23.6565	120
34	21.68770625	16.2365	24.4635	204
35	21.34658333	18.02366667	27.019	167
36	18.65018333	12.95966667	25.912	84
37	15.25785625	11.91833333	20.25433333	6
38	13.81320208	9.098	18.3645	0
39	16.276375	13.6945	18.665	0
40	20.46325833	17.752	24.013	133
41	20.06504386	14.74933333	25.91766667	108
42	22.31879167	19.795	24.76266667	234
43	27.32444167	21.243	32.80266667	240
44	22.131225	18.18966667	27.527	210
45	28.28576667	24.8135	32.6785	240
46	20.55132083	15.248	25.2235	138

Sample #	Mean water temp. [°C] 10 days before sampling	Min. water temp. [°C] 10 days before sampling	Max. water temp. [°C] 10 days before sampling	Time [h] above threshold 10 days before sampling
47	21.45381667	18.5235	23.925	216
48	18.07825417	16.187	20.555	12
49	16.62911458	13.73625	19.9765	0
50	21.1300875	16.2715	25.134	156
51	18.01319375	15.413	20.20725	6
52	18.76641875	15.8945	21.20575	54
53	20.30155	15.947	26.25475	120
54	24.187175	21.29875	28.615	240
55	22.64621875	19.9005	27.1025	228
56	25.57430625	22.8085	29.04925	240
57	21.694175	17.37125	26.7455	192
58	19.50979375	16.594	23.34225	84
59	19.8233375	12.9325	24.189	137
60	15.69001875	8.2215	21.1685	30
61	16.86585465	13.66775	19.80975	0
62	19.44366979	16.499	23.92475	88
63	16.82955521	12.01175	21.98475	16
64	20.00295711	13.2425	25.06025	124
65	19.66484167	16.63475	22.391	97
66	26.56720729	20.5255	32.35575	240
67	23.87790625	19.74325	27.491	238
68	24.78363958	22.4505	28.847	240
69	24.14713542	18.2225	30.74325	216
70	23.70969713	17.70175	27.98625	229
71	23.88919191	23.01025	24.76825	240
72	18.96343125	18.088	19.83875	0
73	14.95357373	11.59525	19.7895	0
74	14.68799167	12.126	18.078	0
75	14.06129271	11.3365	18.47525	0
76	16.98959896	13.194	21.9405	9
77	16.91168438	13.4	19.88575	0
78	22.5662125	19.767	24.74	237
79	19.22340521	16.35425	21.57675	80
80	19.116325	14.8225	22.4545	82
81	22.53122908	17.558	27.627	198
82	17.51176979	12.6965	23.3	27
83	19.23214315	14.4025	23.5315	109
84	21.00157083	18.3595	24.141	170

DA Table B 26: Cooling Tower 2 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 10 days.

Sample #	Mean water temp. [°C] 10 days before sampling	Min. water temp. [°C] 10 days before sampling	Max. water temp. [°C] 10 days before sampling	Time [h] above threshold 10 days before sampling
1	16.74785256	13.66775	19.80975	0
2	19.44366979	16.499	23.92475	88
3	16.82955521	12.01175	21.98475	16
4	20.00295711	13.2425	25.06025	124
5	19.66484167	16.63475	22.391	97
6	23.87790625	19.74325	27.491	238
7	24.78363958	22.4505	28.847	240
8	24.14713542	18.2225	30.74325	216
9	23.70969713	17.70175	27.98625	229
10	23.88919191	23.01025	24.76825	240
11	18.96343125	18.088	19.83875	0
12	14.95357373	11.59525	19.7895	0
13	14.68799167	12.126	18.078	0
14	14.06129271	11.3365	18.47525	0
15	16.98959896	13.194	21.9405	9
16	18.81471563	15.3795	22.03975	58
17	16.91168438	13.4	19.88575	0
18	22.5662125	19.767	24.74	237
19	19.22340521	16.35425	21.57675	80
20	19.116325	14.8225	22.4545	82
21	22.53122908	17.558	27.627	198
22	17.51176979	12.6965	23.3	27
23	19.23214315	14.4025	23.5315	109
24	21.00157083	18.3595	24.141	170

DA Table B 27: Cooling Tower 3 –Microbiological concentrations from the Laboratory of Technical Hygiene, IHPH.

*84/23: Due to lack of water temperature entries from end of November until December the microbiological December values were excluded from correlation analyses.

Legionella Sample #	Sampling Date	Sampling Time	*Legionella* [cfu/100 mL]	Serology	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	HPC Sample #
1	10.01.2012	10:00:00	105	1, 2-14, non			
2	31.01.2012	09:50:00	200	2 - 14			
3	06.03.2012	10:50:00	115	1, 2 - 14			
4	03.04.2012	09:55:00	70	1, 2-14, non			
5	08.05.2012	11:15:00	490	1, 2 - 14			
6	05.06.2012	09:40:00	690	1			
7	10.07.2012	09:50:00	400	1, 2 - 14			
8	07.08.2012	09:25:00	105	2 - 14			
9	04.09.2012	10:10:00	440	1, 2 - 14			
10	01.10.2012	09:50:00	145	2 - 14			
11	06.11.2012	10:00:00	105	1			
12	04.12.2012	10:20:00	1400	1			
13	08.01.2013	09:40:00	240	2 - 14			
14	04.02.2013	09:35:00	550	1			
15	05.03.2013	10:05:00	30	1, 2 - 14			
16	02.04.2013	09:20:00	10	1, 2 - 14			
17	06.05.2013	09:20:00	40	2 - 14			
18	03.06.2013	11:45:00	60	1			
19	02.07.2013	09:35:00	210	1			
20	13.08.2013	09:25:00	150	1			
21	03.09.2013	09:35:00	450	1			
22	24.09.2013	10:15:00	50	1			
23	05.11.2013	12:25:00	2600	1			
24	03.12.2013	13:30:00	3080	2 - 14			
25	14.01.2014	10:25:00	2100	1, 2 - 14			
26	11.02.2014	11:20:00	2400	1, 2 - 14			
27	11.03.2014	11:50:00	100	1			
28	08.04.2014	09:55:00	200	1			
29	06.05.2014	10:50:00	120	1			
30	10.06.2014	11:40:00	180	1, 2 - 14			
31	08.07.2014	13:00:00	105	1			
32	05.08.2014	12:00:00	190	1			
33	02.09.2014	09:35:00	200	1, 2 - 14			
34	07.10.2014	10:30:00	450	1, 2 - 14			
35	04.11.2014	13:30:00	100	1			
36	02.12.2014	12:00:00	100	2 - 14			
37	13.01.2015	11:00:00	<DL				
38	10.02.2015	10:55:00	250	2 - 14			
39	10.03.2015	10:50:00	250	1			
40	07.04.2015	10:10:00	80	2 - 14			
41	05.05.2015	12:50:00	180	1			
42	02.06.2015	10:30:00	170	2 - 14			
43	07.07.2015	10:50:00	350	1			
44	04.08.2015	09:30:00	80	1, 2 - 14			
45	16.09.2015	09:40:00	1050	1, L. sp.			

Legionella Sample #	Sampling Date	Sampling Time	*Legionella* [cfu/100 mL]	Serology	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	HPC Sample #
46	06.10.2015	14:10:00	200	1, 2 - 14			
47	17.11.2015	11:10:00	200	1			
48	01.12.2015	11:00:00	75	1, 2 - 14			
49	12.01.2016	11:06:00	50	2 - 14			
50	02.02.2016	09:50:00	30	1, non			
51	07.03.2016	12:25:00	200	1			
52	06.04.2016	09:25:00	20	2 - 14			
53	11.05.2016	09:10:00	15	2-14, non			
54	07.06.2016	09:58:00	15	2 - 14			
55	04.07.2016	09:15:00	20	2 - 14			
56	02.08.2016	17:10:00	35	2 - 14			
57	06.09.2016	09:35:00	1150	1, L. sp.			
58	04.10.2016	10:33:00	720	2-14, L. sp.			
59	02.11.2016	10:30:00	200	2 - 14			
60	06.12.2016	09:45:00	275	1, 2 - 14			
61	03.01.2017	10:24:00	40	2 - 14	1620	1080	1
62	07.02.2017	09:59:00	505	1, 2 - 14	380	920	2
63	07.03.2017	09:28:00	1680	2-14, L. sp.	83754	94	3
64	04.04.2017	10:06:00	300	1	780	400	4
65	02.05.2017	09:43:00	65	2-14, non	1620	5400	5
66	06.06.2017	08:59:00	55	1			
67	03.07.2017	09:45:00	60	2 - 14	8600	4700	6
68	01.08.2017	10:20:00	120	1, 2 - 14	1080	12420	7
69	05.09.2017	09:20:00	400	1, 2 - 14	1210	2200	8
70	04.10.2017	10:21:00	250	2 - 14	1080	420	9
71	07.11.2017	10:36:00	110	1, 2 - 14	75	73	10
72	05.12.2017	10:15:00	80	1, 2 - 14	130	2800	11
73	09.01.2018	10:35:00	90	1	720	210	12
74	06.02.2018	10:35:00	130	1	200	170	13
75	06.03.2018	11:44:00	70	1, 2 - 14	160	160	14
76	10.04.2018	08:51:00	<DL		3900	730	15
77	08.05.2018	09:07:00	<DL		580	3100	16
78	05.06.2018	09:51:00	40	1, 2 - 14	1300	1060	17
79	03.07.2018	10:29:00	<DL		360	560	18
80	07.08.2018	10:02:00	200	1, 2-14, non	250	590	19
81	04.09.2018	12:15:00	400	2 - 14	430	370	20
82	01.10.2018	11:15:00	5	2 - 14	21600	21600	21
83	05.11.2018	09:15:00	80	1	1130	1800	22
84	03.12.2018	08:52:00	110	1, 2 - 14	1080	1360	23*
	07.01.2019	09:15	40		20	90	24
	04.02.2019	09:37	<DL				
	05.03.2019	08:49	55		380	100	25
	01.04.2019	10:25	30		60	30	26
	06.05.2019	09:52	65		340	150	27
	03.06.2019	09:59	75		830	480	28
	01.07.2019	09:21	95		410	20	29
	05.08.2019	09:20	200		1500	940	30
	02.09.2019	10:04	410		300	220	31
	07.10.2019	09:55	130		460	1810	32

Legionella Sample #	Sampling Date	Sampling Time	*Legionella* [cfu/100 mL]	Serology	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	HPC Sample #
	04.11.2019	12:14	180		600	2700	33
	02.12.2019	09:39	130		630	370	34
	06.01.2020	10:41	85		110	270	
	03.02.2020	10:38	30		130	130	
	02.03.2020	09:33	90		110	110	
	06.04.2020	09:30	50		6800	2300	
	04.05.2020	10:20	450		90	110	
	02.06.2020	10:00	85		100	110	
	06.07.2020	09:51	120		380	130	
	03.08.2020	09:40	100		760	1190	
	07.09.2020	10:30	25		40	70	

DA Table B 28: Cooling Tower 3 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 5 days.

Sample #	Biocide dosages 5 days before sampling	Biocide dosages 5 days after sampling	Biocide dosages 5 days before and after sampling	Ratio of dosages 5 days after and before sampling	Mean mV 5 days before sampling	Time [h] below threshold 5 days before sampling
1	22	20	42	0.909090909	408.09	68
2	30	30	60	1	434.5413333	47
3	21	31	52	1.476190476	448.2741667	42
4	20	33	53	1.65	413.6270833	61
5	39	39	78	1	421.1176667	54
6	21	20	41	0.952380952	412.3711667	66
7	39	23	62	0.58974359	414.5131667	59
8	32	35	67	1.09375	399.5990833	79
9	29	39	68	1.344827586	395.37225	86
10	22	25	47	1.136363636	406.70875	64
11	43	41	84	0.953488372	414.2876471	53
12	31	37	68	1.193548387	414.5184167	56
13	28	44	72	1.571428571	432.1596814	45
14	31	41	72	1.322580645	421.0095985	40
15	18	19	37	1.055555556	460.7354106	9
16	9	11	20	1.222222222	401.4807152	80
17	17	17	34	1	455.8101026	0
18	23	24	47	1.043478261	439.649795	35
19	14	19	33	1.357142857	397.3770851	81
20	22	28	50	1.272727273	431.2383301	48
21	19	19	38	1	436.9397163	5
22	33	35	68	1.060606061	428.8235229	54
23	29	17	46	0.586206897	404.107947	62
24	30	21	51	0.7	426.3242732	38
25	19	19	38	1	437.3229312	2
26	27	25	52	0.925925926	371.3173138	86
27	50	40	90	0.8	452.7305094	3
28	31	27	58	0.870967742	440.3048243	6
29	26	32	58	1.230769231	451.8537539	1
30	25	24	49	0.96	468.9613859	7
31	26	29	55	1.115384615	466.6971607	8
32	31	27	58	0.870967742	472.465162	10
33	21	23	44	1.095238095	477.4211889	2
34	33	31	64	0.939393939	468.3453222	7
35	25	23	48	0.92	470.9662715	9
36	27	32	59	1.185185185	467.5718792	18
37	43	44	87	1.023255814	473.5363644	8
38	28	21	49	0.75	473.3485446	5
39	26	21	47	0.807692308	436.0432482	38
40	19	19	38	1	473.480837	3
41	16	19	35	1.1875	467.6119766	13
42	18	15	33	0.833333333	466.8969017	25
43	10	16	26	1.6	417.8127764	61
44	18	17	35	0.944444444	463.0211568	15
45	16	18	34	1.125	472.9884405	3
46	20	19	39	0.95	443.4355634	40
47	16	12	28	0.75	455.9764476	6

Sample #	Biocide dosages 5 days before sampling	Biocide dosages 5 days after sampling	Biocide dosages 5 days before and after sampling	Ratio of dosages 5 days after and before sampling	Mean mV 5 days before sampling	Time [h] below threshold 5 days before sampling
48	19	19	38	1	448.5914507	20
49	7	5	12	0.714285714	430.6892351	9
50	19	19	38	1	464.8925072	2
51	17	20	37	1.176470588	419.0896133	6
52	42	38	80	0.904761905	412.1807302	56
53	25	19	44	0.76	422.5554354	47
54	25	22	47	0.88	435.0485446	39
55	26	30	56	1.153846154	423.75467	42
56	23	21	44	0.913043478	420.9066279	44
57	21	24	45	1.142857143	419.7302007	55
58	23	20	43	0.869565217	420.0226613	40
59	20	20	40	1	418.8702097	44
60	22	27	49	1.227272727	432.2101718	40
61	21	20	41	0.952380952	412.2104307	56
62	30	32	62	1.066666667	409.6202759	51
63	20	20	40	1	412.1697802	15
64	24	20	44	0.833333333	425.9426086	35
65	19	18	37	0.947368421	431.8717791	5
66	21	19	40	0.904761905	425.8632373	40
67	19	20	39	1.052631579	434.5204061	12
68	19	18	37	0.947368421	442.9392504	25
69	17	21	38	1.235294118	415.7237063	44
70	18	18	36	1	434.6292973	7
71	0	0	0	0	422.3882767	0
72	0	13	13	13	428.7981366	0
73	21	16	37	0.761904762	428.9703626	0
74	18	20	38	1.111111111	448.9625313	0
75	19	19	38	1	439.3251531	0
76	24	28	52	1.166666667	402.7559891	55
77	17	34	51	2	428.7347529	17
78	26	36	62	1.384615385	440.9831355	1
79	25	30	55	1.2	441.0741849	1
80	24	43	67	1.791666667	443.4514094	2
81	20	4	24	0.2	421.2009257	45
82	15	37	52	2.466666667	410.3009071	77
83	36	20	56	0.555555556	440.832237	3
84	26	20	46	0.769230769	456.5567149	0

DA Table B 29: Cooling Tower 3 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 5 days.

Sample #	Biocide dosages 5 days before sampling	Biocide dosages 5 days after sampling	Biocide dosages 5 days before and after sampling	Ratio of dosages 5 days after and before sampling	Mean mV 5 days before sampling	Time [h] below threshold 5 days before sampling
1	21	20	41	0.952380952	412.2104307	56
2	30	32	62	1.066666667	409.6202759	51
3	20	20	40	1	412.1697802	15
4	24	20	44	0.833333333	425.9426086	35
5	19	18	37	0.947368421	431.8717791	5
6	19	20	39	1.052631579	434.5204061	12
7	19	18	37	0.947368421	442.9392504	25
8	17	21	38	1.235294118	415.7237063	44
9	18	18	36	1	434.6292973	7
10	0	0	0	0	422.3882767	0
11	0	13	13	13	428.7981366	0
12	21	16	37	0.761904762	428.9703626	0
13	18	20	38	1.111111111	448.9625313	0
14	19	19	38	1	439.3251531	0
15	24	28	52	1.166666667	402.7559891	55
16	17	34	51	2	428.7347529	17
17	26	36	62	1.384615385	440.9831355	1
18	25	30	55	1.2	441.0741849	1
19	24	43	67	1.791666667	443.4514094	2
20	20	4	24	0.2	421.2009257	45
21	15	37	52	2.466666667	410.3009071	77
22	36	20	56	0.555555556	440.832237	3
23	26	20	46	0.769230769	456.5567149	0
24	34	37	71	1.088235294	443.8744665	0
25	39	24	63	0.615384615	449.2098752	2
26	38	42	80	1.105263158	446.4380627	2
27	45	32	77	0.711111111	449.179248	4
28	24	44	68	1.833333333	448.977551	0
29	33	48	81	1.454545455	440.6291072	3
30	36	51	87	1.416666667	438.3134933	1
31	44	29	73	0.659090909	440.6345286	4
32	41	39	80	0.951219512	449.694989	2
33	42	33	75	0.785714286	453.6928551	2
34	45	42	87	0.933333333	465.9547798	2

DA Table B 30: Cooling Tower 3 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 7 days.

Sample #	Biocide dosages 7 days before sampling	Biocide dosages 7 days after sampling	Biocide dosages 7 days before and after sampling	Ratio of dosages 7 days after and before sampling	Mean mV 7 days before sampling	Time [h] below threshold 7 days before sampling
1	30	28	58	0.933333333	407.7776647	97
2	43	36	79	0.837209302	432.9220833	65
3	30	47	77	1.566666667	448.86875	56
4	34	49	83	1.441176471	411.49375	88
5	60	50	110	0.833333333	420.7095833	74
6	29	28	57	0.965517241	407.5229167	102
7	51	33	84	0.647058824	414.3991667	86
8	44	43	87	0.977272727	400.0558333	109
9	41	47	88	1.146341463	397.5445833	116
10	31	33	64	1.064516129	405.605	94
11	62	53	115	0.85483871	413.2510778	75
12	40	49	89	1.225	416.185	76
13	40	61	101	1.525	430.7540439	66
14	39	59	98	1.512820513	417.5862125	71
15	26	26	52	1	457.2832547	19
16	13	16	29	1.230769231	403.821468	84
17	23	23	46	1	455.9318986	0
18	30	32	62	1.066666667	445.9993344	36
19	21	27	48	1.285714286	411.4861937	97
20	33	37	70	1.121212121	431.6558335	67
21	26	23	49	0.884615385	443.701579	5
22	46	43	89	0.934782609	427.2946999	78
23	45	23	68	0.511111111	406.021541	82
24	40	23	63	0.575	431.4613066	43
25	28	35	63	1.25	438.4261467	4
26	32	28	60	0.875	360.9291099	128
27	70	58	128	0.828571429	451.7625433	4
28	51	41	92	0.803921569	439.3470828	9
29	39	44	83	1.128205128	446.0606617	4
30	34	35	69	1.029411765	473.2733895	9
31	33	41	74	1.242424242	460.3992403	20
32	46	37	83	0.804347826	470.9446651	17
33	30	36	66	1.2	479.5528317	3
34	47	50	97	1.063829787	467.0594228	7
35	34	32	66	0.941176471	472.0648615	13
36	37	42	79	1.135135135	472.5885929	18
37	51	54	105	1.058823529	479.2696568	8
38	38	36	74	0.947368421	475.81593	9
39	30	30	60	1	418.6722339	74
40	26	29	55	1.115384615	462.6225584	20
41	24	25	49	1.041666667	470.3896075	14
42	26	24	50	0.923076923	471.9187707	26
43	19	22	41	1.157894737	421.0923891	82
44	24	24	48	1	464.9895292	15
45	24	26	50	1.083333333	472.4913299	12
46	29	24	53	0.827586207	446.5084773	51
47	24	20	44	0.833333333	455.079095	17

Sample #	Biocide dosages 7 days before sampling	Biocide dosages 7 days after sampling	Biocide dosages 7 days before and after sampling	Ratio of dosages 7 days after and before sampling	Mean mV 7 days before sampling	Time [h] below threshold 7 days before sampling
48	26	24	50	0.923076923	451.3997996	26
49	10	12	22	1.2	434.7175894	10
50	27	25	52	0.925925926	461.9204714	9
51	25	27	52	1.08	415.4410771	25
52	52	47	99	0.903846154	410.3585416	84
53	36	26	62	0.722222222	424.7552843	61
54	34	29	63	0.852941176	434.5795749	57
55	38	40	78	1.052631579	422.4006669	64
56	35	31	66	0.885714286	422.2971905	63
57	29	32	61	1.103448276	419.4891239	79
58	36	28	64	0.777777778	423.0425609	58
59	29	28	57	0.965517241	419.720401	64
60	30	35	65	1.166666667	430.2621053	60
61	30	29	59	0.966666667	415.3382407	70
62	37	41	78	1.108108108	410.8043393	64
63	28	28	56	1	410.3314745	40
64	33	29	62	0.878787879	421.4489094	59
65	26	25	51	0.961538462	435.2463032	5
66	29	26	55	0.896551724	422.4654129	63
67	26	29	55	1.115384615	433.4734553	20
68	27	27	54	1	443.9159602	36
69	26	30	56	1.153846154	410.9881085	73
70	26	26	52	1	434.6775215	7
71	0	0	0	0	422.1593532	0
72	0	23	23	23	428.5692133	0
73	29	16	45	0.551724138	438.2196105	1
74	25	30	55	1.2	451.4432157	3
75	25	28	53	1.12	440.2705525	0
76	33	42	75	1.272727273	408.1609074	76
77	26	48	74	1.846153846	430.9247208	17
78	37	50	87	1.351351351	440.7968815	3
79	34	39	73	1.147058824	442.4050289	2
80	35	63	98	1.8	443.3672941	4
81	42	9	51	0.214285714	428.645054	45
82	15	47	62	3.133333333	405.2049352	125
83	51	32	83	0.62745098	439.4160685	3
84	31	33	64	1.064516129	450.4502089	0

DA Table B 31: Cooling Tower 3 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 7 days.

Sample #	Biocide dosages 7 days before sampling	Biocide dosages 7 days after sampling	Biocide dosages 7 days before and after sampling	Ratio of dosages 7 days after and before sampling	Mean mV 7 days before sampling	Time [h] below threshold 7 days before sampling
1	30	29	59	0.966666667	415.3382407	70
2	37	41	78	1.108108108	410.8043393	64
3	28	28	56	1	410.3314745	40
4	33	29	62	0.878787879	421.4489094	59
5	26	25	51	0.961538462	435.2463032	5
6	26	29	55	1.115384615	433.4734553	20
7	27	27	54	1	443.9159602	36
8	26	30	56	1.153846154	410.9881085	73
9	26	26	52	1	434.6775215	7
10	0	0	0	0	422.1593532	0
11	0	23	23	23	428.5692133	0
12	29	16	45	0.551724138	438.2196105	1
13	25	30	55	1.2	451.4432157	3
14	25	28	53	1.12	440.2705525	0
15	33	42	75	1.272727273	408.1609074	76
16	26	48	74	1.846153846	430.9247208	17
17	37	50	87	1.351351351	440.7968815	3
18	34	39	73	1.147058824	442.4050289	2
19	35	63	98	1.8	443.3672941	4
20	42	9	51	0.214285714	428.645054	45
21	15	47	62	3.133333333	405.2049352	125
22	51	32	83	0.62745098	439.4160685	3
23	31	33	64	1.064516129	450.4502089	0
24	43	48	91	1.11627907	445.4220648	0
25	58	39	97	0.672413793	455.7906663	3
26	53	58	111	1.094339623	451.1576586	4
27	66	49	115	0.742424242	450.6446807	6
28	32	56	88	1.75	449.1237904	0
29	46	67	113	1.456521739	443.612268	5
30	57	64	121	1.122807018	438.7077431	2
31	58	49	107	0.844827586	439.3800016	4
32	63	62	125	0.984126984	452.5877469	3
33	60	46	106	0.766666667	455.0658511	2
34	64	60	124	0.9375	463.1415801	4

DA Table B 32: Cooling Tower 3 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 10 days.

Sample #	Biocide dosages 10 days before sampling	Biocide dosages 10 days after sampling	Biocide dosages 10 days before and after sampling	Ratio of dosages 10 days after and before sampling	Mean mV 10 days before sampling	Time [h] below threshold 10 days before sampling
1	40	35	75	0.875	408.6214222	128
2	56	48	104	0.857142857	423.1354167	113
3	40	69	109	1.725	439.3450833	96
4	48	74	122	1.541666667	405.800251	141
5	90	71	161	0.788888889	421.3932917	101
6	41	43	84	1.048780488	404.79425	156
7	67	55	122	0.820895522	414.81125	127
8	67	55	122	0.820895522	403.0535833	147
9	54	65	119	1.203703704	395.9240833	173
10	47	45	92	0.957446809	402.3340417	145
11	82	73	155	0.890243902	410.598125	117
12	57	68	125	1.192982456	419.0762917	101
13	57	85	142	1.49122807	432.285278	86
14	50	88	138	1.76	420.2054241	86
15	39	36	75	0.923076923	451.5420096	38
16	23	27	50	1.173913043	413.8622157	100
17	32	35	67	1.09375	454.3317064	0
18	40	44	84	1.1	452.444466	36
19	34	38	72	1.117647059	419.66891	126
20	50	52	102	1.04	431.3294285	95
21	37	38	75	1.027027027	442.7494066	20
22	67	55	122	0.820895522	427.0222136	112
23	69	28	97	0.405797101	410.9406654	105
24	57	35	92	0.614035088	433.8046585	49
25	40	61	101	1.525	437.298281	5
26	43	48	91	1.11627907	359.8757328	189
27	99	85	184	0.858585859	454.4953298	7
28	82	62	144	0.756097561	438.0845666	12
29	56	56	112	1	440.3982449	9
30	47	51	98	1.085106383	478.5620556	12
31	45	58	103	1.288888889	463.8645569	23
32	67	54	121	0.805970149	469.6648546	27
33	43	51	94	1.186046512	482.8821945	5
34	65	79	144	1.215384615	464.0428387	12
35	52	45	97	0.865384615	472.7478196	20
36	52	56	108	1.076923077	470.659341	23
37	62	67	129	1.080645161	482.4502945	8
38	51	54	105	1.058823529	480.7080128	19
39	33	47	80	1.424242424	395.0526398	136
40	41	45	86	1.097560976	466.0667197	24
41	36	29	65	0.805555556	470.3288844	15
42	32	36	68	1.125	472.5913464	26
43	31	33	64	1.064516129	427.0931	112
44	36	35	71	0.972222222	465.071909	15
45	36	37	73	1.027777778	464.7912618	37
46	40	29	69	0.725	454.2028848	52

Sample #	Biocide dosages 10 days before sampling	Biocide dosages 10 days after sampling	Biocide dosages 10 days before and after sampling	Ratio of dosages 10 days after and before sampling	Mean mV 10 days before sampling	Time [h] below threshold 10 days before sampling
47	35	31	66	0.885714286	455.5335486	17
48	37	35	72	0.945945946	452.3328114	39
49	13	23	36	1.769230769	436.1129111	10
50	38	30	68	0.789473684	462.135193	16
51	35	40	75	1.142857143	415.2729305	26
52	65	58	123	0.892307692	407.4520383	129
53	51	38	89	0.745098039	425.7348342	86
54	51	40	91	0.784313725	432.6394863	83
55	51	53	104	1.039215686	423.3279325	89
56	49	43	92	0.87755102	424.5645198	93
57	40	45	85	1.125	425.3104602	95
58	48	42	90	0.875	422.3893147	90
59	42	40	82	0.952380952	418.6689082	97
60	42	49	91	1.166666667	430.8464433	66
61	42	41	83	0.976190476	413.51841	103
62	49	56	105	1.142857143	413.0969582	64
63	41	44	85	1.073170732	409.5379094	76
64	45	43	88	0.955555556	416.9838279	98
65	37	36	73	0.972972973	431.3076565	14
66	45	38	83	0.844444444	419.3038119	102
67	37	40	77	1.081081081	432.4315884	36
68	39	39	78	1	447.5751958	42
69	37	42	79	1.135135135	407.0345711	117
70	38	39	77	1.026315789	431.8820757	13
71	0	0	0	0	421.8111985	0
72	0	36	36	36	428.225828	0
73	40	23	63	0.575	444.2002054	1
74	37	41	78	1.108108108	448.6908276	12
75	31	40	71	1.290322581	437.6782117	0
76	45	63	108	1.4	410.7499172	108
77	45	66	111	1.466666667	431.4261735	19
78	56	68	124	1.214285714	440.6042047	4
79	49	54	103	1.102040816	442.2536484	5
80	48	94	142	1.958333333	441.3454299	5
81	69	17	86	0.246376812	432.8663088	48
82	15	60	75	4	400.0173602	197
83	76	46	122	0.605263158	440.8855652	3
84	31	48	79	1.548387097	440.0761448	0

DA Table B 33: Cooling Tower 3 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 10 days.

Sample #	Biocide dosages 10 days before sampling	Biocide dosages 10 days after sampling	Biocide dosages 10 days before and after sampling	Ratio of dosages 10 days after and before sampling	Mean mV 10 days before sampling	Time [h] below threshold 10 days before sampling
1	42	41	83	0.976190476	413.51841	103
2	49	56	105	1.142857143	413.0969582	64
3	41	44	85	1.073170732	409.5379094	76
4	45	43	88	0.955555556	416.9838279	98
5	37	36	73	0.972972973	431.3076565	14
6	37	40	77	1.081081081	432.4315884	36
7	39	39	78	1	447.5751958	42
8	37	42	79	1.135135135	407.0345711	117
9	38	39	77	1.026315789	431.8820757	13
10	0	0	0	0	421.8111985	0
11	0	36	36	36	428.225828	0
12	40	23	63	0.575	444.2002054	1
13	37	41	78	1.108108108	448.6908276	12
14	31	40	71	1.290322581	437.6782117	0
15	45	63	108	1.4	410.7499172	108
16	45	66	111	1.466666667	431.4261735	19
17	56	68	124	1.214285714	440.6042047	4
18	49	54	103	1.102040816	442.2536484	5
19	48	94	142	1.958333333	441.3454299	5
20	69	17	86	0.246376812	432.8663088	48
21	15	60	75	4	400.0173602	197
22	76	46	122	0.605263158	440.8855652	3
23	31	48	79	1.548387097	440.0761448	0
24	56	64	120	1.142857143	447.772543	0
25	85	59	144	0.694117647	458.1817762	3
26	79	79	158	1	451.8129593	5
27	95	77	172	0.810526316	450.6202621	7
28	52	76	128	1.461538462	447.8300236	0
29	68	93	161	1.367647059	446.0503207	5
30	90	77	167	0.855555556	439.3569981	2
31	85	80	165	0.941176471	443.3878605	5
32	93	89	182	0.956989247	452.4876928	5
33	88	64	152	0.727272727	455.8270262	2
34	84	73	157	0.869047619	460.7131744	6

DA Table B 34: Cooling Tower 3 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 5 days.

Sample #	Mean water temp. [°C] 5 days before sampling	Min. water temp. [°C] 5 days before sampling	Max. water temp. [°C] 5 days before sampling	Time [h] above threshold 5 days before sampling
1	18.3423	16.917	19.9	0
2	17.41605	15.744	19.437	0
3	18.2103	17.033	19.074	0
4	18.40485	16.597	20.116	6
5	18.788	16.708	21.833	30
6	19.6054	16.935	23.165	42
7	24.0921	15.703	26.402	114
8	23.52745	21.411	25.563	120
9	21.41015	17.713	23.578	84
10	19.85165	17.16	21.391	78
11	18.1733	16.483	19.255	0
12	17.32175	15.679	19.161	0
13	19.3934	17.38	21.649	42
14	17.7973	15.822	21.137	6
15	17.88325	16.571	19.442	0
16	17.54375	16.187	19.61	0
17	19.78575	17.322	22.717	66
18	19.4465	15.846	22.548	36
19	21.75685	19.025	23.68	108
20	22.5392	19.222	24.309	114
21	22.3583	20.072	23.925	120
22	18.78315	15.758	20.534	12
23	18.04925	16.501	20.193	6
24	17.4241	11.31	19.088	0
25	18.08395	16.294	20.188	6
26	18.23815	16.505	20.556	12
27	18.72295	15.979	20.612	24
28	22.02635	19.331	23.555	114
29	18.3293	15.928	22.514	24
30	24.59605	19.717	27.937	114
31	25.79415	23.271	27.994	120
32	25.293	23.059	26.926	114
33	24.3756	21.417	26.214	120
34	25.0856	23.277	26.363	120
35	23.09355	21.354	24.446	120
36	17.7814	16.611	18.954	0
37	19.04710526	16.337	22.202	18
38	18.81795	15.991	20.308	18
39	18.98435	15.565	20.981	18
40	18.1745	16.566	20.336	6
41	19.02033333	16.656	22.474	36
42	19.01985	17.097	21.745	18
43	26.7462	22.548	29.443	120
44	21.82225	18.397	25.974	96
45	22.0109	18.898	24.475	96
46	19.65025	15.588	23.069	66

Sample #	Mean water temp. [°C] 5 days before sampling	Min. water temp. [°C] 5 days before sampling	Max. water temp. [°C] 5 days before sampling	Time [h] above threshold 5 days before sampling
47	21.5377	20.747	22.549	120
48	21.37673684	19.58	22.972	102
49	19.01365	16.302	20.799	30
50	16.393	16.393	16.393	0
51	19.35775	17.337	20.502	36
52	18.61625	16.945	20.215	6
53	19.65435	17.923	21.023	48
54	23.0729	21.286	24.275	120
55	22.0614	19.81	24.14	102
56	23.22685	20.705	24.863	120
57	22.2329	17.897	24.476	108
58	19.935	16.9	22.297	60
59	19.2337	16.799	21.955	48
60	14.46535	10.32	19.504	0
61	14.80216667	11.083	16.698	0
62	17.39180833	15.739	20.732	3
63	18.13263333	16.034	20.501	6
64	19.41863333	15.926	21.64	38
65	17.48204167	15.991	19.647	0
66	21.40133333	17.29	24.036	103
67	21.689025	17.386	24.493	110
68	22.02628333	20.071	24.122	120
69	20.53765833	18.54	22.088	84
70	21.88394017	18.708	24.037	109
71	20.44770833	20.208	20.687	120
72	17.74126667	17.502	17.981	0
73	18.31901667	16.55	20.594	13
74	17.47605	14.799	21.735	13
75	20.428675	18.575	23.354	78
76	19.84728333	16.738	22.385	47
77	19.70318333	18.052	21.579	43
78	22.76505	20.122	25.4	120
79	20.5109	18.03	22.67	82
80	22.34035833	18.812	24.9	111
81	20.025325	16.942	22.212	68
82	18.98910833	17.331	20.05	5
83	19.304275	15.694	21.441	41

DA Table B 35: Cooling Tower 3 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 5 days.

Sample #	Mean water temp. [°C] 5 days before sampling	Min. water temp. [°C] 5 days before sampling	Max. water temp. [°C] 5 days before sampling	Time [h] above threshold 5 days before sampling
1	14.80216667	11.083	16.698	0
2	17.39180833	15.739	20.732	3
3	18.13263333	16.034	20.501	6
4	19.41863333	15.926	21.64	38
5	17.48204167	15.991	19.647	0
6	21.689025	17.386	24.493	110
7	22.02628333	20.071	24.122	120
8	20.53765833	18.54	22.088	84
9	21.88394017	18.708	24.037	109
10	20.44770833	20.208	20.687	120
11	17.74126667	17.502	17.981	0
12	18.31901667	16.55	20.594	13
13	17.47605	14.799	21.735	13
14	20.428675	18.575	23.354	78
15	19.84728333	16.738	22.385	47
16	19.70318333	18.052	21.579	43
17	22.76505	20.122	25.4	120
18	20.5109	18.03	22.67	82
19	22.34035833	18.812	24.9	111
20	20.025325	16.942	22.212	68
21	18.98910833	17.331	20.05	5
22	19.304275	15.694	21.441	41

DA Table B 36: Cooling Tower 3 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 7 days.

Sample #	Mean water temp. [°C] 7 days before sampling	Min. water temp. [°C] 7 days before sampling	Max. water temp. [°C] 7 days before sampling	Time [h] above threshold 7 days before sampling
1	18.30228571	16.917	19.9	0
2	17.34596429	15.715	19.437	0
3	18.53214286	17.033	19.624	0
4	18.74282143	16.597	21.884	24
5	19.74239286	16.708	23.934	78
6	20.05721429	16.935	23.165	84
7	24.27157143	15.703	26.402	162
8	23.64546429	21.411	25.605	168
9	21.91867857	17.713	24.882	132
10	20.21585714	17.16	22.784	126
11	18.13842857	16.483	19.255	0
12	17.33928571	15.679	19.161	0
13	19.37117857	17.38	21.649	42
14	18.35253571	15.822	21.415	24
15	17.98914286	16.571	19.817	0
16	17.54035714	16.187	19.61	0
17	19.33942857	16.43	22.717	72
18	19.57553571	15.846	22.548	54
19	21.31071429	17.61	23.68	138
20	22.90771429	19.222	25.573	162
21	22.36378571	20.072	23.925	168
22	18.70703571	15.758	20.534	12
23	18.03117857	14.775	20.242	12
24	17.84921429	11.31	19.668	0
25	18.40664286	16.294	20.188	12
26	18.13033333	16.505	20.556	12
27	18.34396429	15.916	20.612	24
28	21.77717857	19.206	23.555	150
29	19.60528571	15.928	23.559	72
30	23.61896429	18.551	27.937	156
31	24.35467857	11.616	27.994	162
32	25.47722222	23.059	28.083	162
33	23.79346429	20.263	26.214	168
34	25.1815	23.277	26.363	168
35	22.23864286	17.485	24.446	150
36	17.96678571	16.611	20.343	6
37	18.96014815	16.337	22.202	18
38	18.56928571	15.991	20.308	18
39	19.14414286	15.565	20.981	24
40	18.04189286	16.566	20.336	6
41	18.74396154	16.265	22.474	36
42	18.95371429	17.097	21.745	18
43	26.16432143	22.415	29.443	168
44	21.65442857	18.397	25.974	138
45	21.67960714	18.541	24.475	132
46	19.75992857	15.588	23.069	96

Sample #	Mean water temp. [°C] 7 days before sampling	Min. water temp. [°C] 7 days before sampling	Max. water temp. [°C] 7 days before sampling	Time [h] above threshold 7 days before sampling
47	21.17767857	18.816	22.549	138
48	21.22003704	16.507	23.153	138
49	18.66175	16.302	20.799	30
50	17.43388889	16.266	18.845	0
51	19.42253571	16.835	20.669	60
52	18.73803571	16.945	20.215	12
53	19.41935714	17.455	21.023	48
54	22.88860714	20.856	24.53	168
55	21.81325	18.812	24.14	144
56	23.40328571	20.705	24.863	168
57	21.93889286	17.354	24.476	144
58	20.28885714	16.9	22.788	96
59	19.54685714	16.799	21.955	78
60	13.96242857	9.023	19.504	0
61	14.9512973	11.083	18.877	0
62	17.65879167	15.739	20.732	8
63	18.00327381	16.034	20.501	6
64	19.48031548	15.926	21.64	48
65	17.3165119	14.628	19.647	0
66	21.84733929	17.29	25.682	148
67	21.43131548	17.386	24.493	143
68	22.02240476	20.071	24.122	168
69	21.60620833	18.54	26.016	132
70	21.91998788	18.708	24.037	150
71	20.54438095	20.208	20.881	168
72	17.83791667	17.502	18.174	0
73	18.47635119	16.55	20.594	13
74	17.75871429	14.799	21.735	13
75	20.49289286	18.575	23.354	126
76	19.92969643	16.738	22.385	74
77	19.464125	17.402	21.579	47
78	23.0655119	20.122	25.4	168
79	20.54110119	18.03	22.67	118
80	22.29322619	18.812	24.9	159
81	20.20152976	16.942	22.212	103
82	19.4157619	17.331	20.871	53
83	19.10663095	15.694	21.441	43

DA Table B 37: Cooling Tower 3 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 7 days.

Sample #	Mean water temp. [°C] 7 days before sampling	Min. water temp. [°C] 7 days before sampling	Max. water temp. [°C] 7 days before sampling	Time [h] above threshold 7 days before sampling
1	14.9512973	11.083	18.877	0
2	17.65879167	15.739	20.732	8
3	18.00327381	16.034	20.501	6
4	19.48031548	15.926	21.64	48
5	17.3165119	14.628	19.647	0
6	21.43131548	17.386	24.493	143
7	22.02240476	20.071	24.122	168
8	21.60620833	18.54	26.016	132
9	21.91998788	18.708	24.037	150
10	20.54438095	20.208	20.881	168
11	17.83791667	17.502	18.174	0
12	18.47635119	16.55	20.594	13
13	17.75871429	14.799	21.735	13
14	20.49289286	18.575	23.354	126
15	19.92969643	16.738	22.385	74
16	19.464125	17.402	21.579	47
17	23.0655119	20.122	25.4	168
18	20.54110119	18.03	22.67	118
19	22.29322619	18.812	24.9	159
20	20.20152976	16.942	22.212	103
21	19.4157619	17.331	20.871	53
22	19.10663095	15.694	21.441	43

DA Table B 38: Cooling Tower 3 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 20 °C, observation period: 10 days.

Sample #	Mean water temp. [°C] 10 days before sampling	Min. water temp. [°C] 10 days before sampling	Max. water temp. [°C] 10 days before sampling	Time [h] above threshold 10 days before sampling
1	18.25021053	16.125	19.9	0
2	17.199475	15.715	19.798	0
3	18.322525	16.393	19.624	0
4	18.696325	15.386	22.112	36
5	20.1472	16.708	23.934	132
6	20.88655	16.935	24.69	156
7	23.782525	15.703	26.402	228
8	23.4775	20.914	25.605	240
9	22.320275	17.713	24.882	204
10	19.9115	15.917	22.784	138
11	18.05215	15.695	19.838	0
12	17.677075	15.679	20.339	6
13	19.399125	17.38	21.649	48
14	18.359275	15.822	21.415	30
15	18.243225	16.571	19.817	0
16	17.041	12.88	19.61	0
17	18.90555	15.92	22.717	78
18	19.342525	15.846	22.548	54
19	21.570775	17.61	23.993	210
20	23.442075	19.222	26.301	234
21	22.46225	20.072	24.833	240
22	18.4416	15.758	21.722	24
23	18.8958	14.775	24.015	59
24	17.8617	11.31	20.275	12
25	18.47305	16.218	20.77	36
26	18.18141026	16.251	20.556	12
27	18.111275	15.916	20.612	24
28	21.221825	17.122	23.555	186
29	20.1466	15.928	23.559	144
30	22.377325	17.423	27.937	186
31	23.90285	11.616	27.994	234
32	25.90228205	23.059	28.083	234
33	23.241475	19.172	26.214	234
34	25.1182	21.958	26.522	240
35	22.0315	17.485	24.446	209
36	18.411025	16.39	22.107	36
37	18.65553846	15.906	22.202	18
38	18.51115	15.991	20.308	18
39	18.930525	15.52	20.981	30
40	18.30225	16.566	20.34	18
41	18.44055263	15.842	22.474	36
42	18.97365	16.955	21.745	24
43	25.013575	18.493	29.443	234
44	21.98925	18.397	25.974	210
45	21.75385	18.541	24.475	198
46	19.8507	15.588	23.069	144

Sample #	Mean water temp. [°C] 10 days before sampling	Min. water temp. [°C] 10 days before sampling	Max. water temp. [°C] 10 days before sampling	Time [h] above threshold 10 days before sampling
47	20.628275	17.059	22.812	156
48	20.98025641	16.507	23.153	180
49	18.62175	16.302	20.799	42
50	18.44257143	16.266	20.969	30
51	19.74835	16.835	22.371	108
52	18.861325	16.945	21.247	18
53	19.140575	16.546	21.259	54
54	22.57915	20.418	24.53	240
55	21.943375	18.812	26.342	210
56	23.677275	20.705	25.455	240
57	22.211375	17.354	25.206	210
58	20.4328	16.9	23.705	138
59	19.33315	16.086	21.955	84
60	13.9929	9.023	19.504	0
61	15.3515	11.083	20.48	12
62	18.35967083	15.059	22.989	55
63	18.1124625	16.034	20.501	6
64	19.24405021	15.926	21.64	64
65	17.44357083	14.628	20.405	6
66	22.78499167	17.29	27.709	220
67	21.60308333	17.386	24.493	215
68	21.93671667	20.071	24.568	240
69	21.93520417	18.54	26.016	204
70	21.39788186	18.708	24.037	189
71	20.69139004	20.208	21.175	240
72	17.98290417	17.502	18.464	0
73	18.62147005	16.332	22.284	26
74	18.2025125	14.799	21.735	19
75	20.40776667	18.575	23.354	164
76	20.04546667	16.738	22.385	130
77	19.503225	17.21	21.943	77
78	22.97904167	20.122	25.4	240
79	20.44312917	18.03	22.67	158
80	22.47317083	18.812	24.9	231
81	20.20344167	16.942	22.212	152
82	19.9792125	17.331	21.614	125
83	18.83692531	15.694	21.441	43

DA Table B 39: Cooling Tower 3 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 20 °C, observation period: 10 days.

Sample #	Mean water temp. [°C] 10 days before sampling	Min. water temp. [°C] 10 days before sampling	Max. water temp. [°C] 10 days before sampling	Time [h] above threshold 10 days before sampling
1	15.3515	11.083	20.48	12
2	18.35967083	15.059	22.989	55
3	18.1124625	16.034	20.501	6
4	19.24405021	15.926	21.64	64
5	17.44357083	14.628	20.405	6
6	21.60308333	17.386	24.493	215
7	21.93671667	20.071	24.568	240
8	21.93520417	18.54	26.016	204
9	21.39788186	18.708	24.037	189
10	20.69139004	20.208	21.175	240
11	17.98290417	17.502	18.464	0
12	18.62147005	16.332	22.284	26
13	18.2025125	14.799	21.735	19
14	20.40776667	18.575	23.354	164
15	20.04546667	16.738	22.385	130
16	19.503225	17.21	21.943	77
17	22.97904167	20.122	25.4	240
18	20.44312917	18.03	22.67	158
19	22.47317083	18.812	24.9	231
20	20.20344167	16.942	22.212	152
21	19.9792125	17.331	21.614	125
22	18.83692531	15.694	21.441	43

DA Table B 40: Cooling Tower 4 – Microbiological concentrations from the Laboratory of Technical Hygiene, IHPH.

Legionella Sample #	Sampling Date	Sampling Time	*Legionella* [cfu/100 mL]	Serology	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	HPC Sample #
1	10.01.2012	11:20:00	<DL				
2	31.01.2012	11:10:00	40	2 - 14			
3	06.03.2012	11:55:00	50	2 - 14			
4	03.04.2012	11:20:00	1500	2-14, L. sp.			
5	08.05.2012	12:40:00	150	2 - 14			
6	05.06.2012	10:40:00	<DL				
7	10.07.2012	11:10:00	400	2 - 14			
8	07.08.2012	10:20:00	100	2 - 14			
9	04.09.2012	11:30:00	200	2 - 14			
10	01.10.2012	11:15:00	60	2 - 14			
11	06.11.2012	11:10:00	200	2 - 14			
12	04.12.2012	12:30:00	10	1			
13	08.01.2013	11:10:00	900	2 - 14			
14	04.02.2013	12:05:00	<DL				
15	05.03.2013	11:25:00	10	2 - 14			
16	02.04.2013	10:30:00	<DL				
17	06.05.2013	10:45:00	50	2 - 14			
18	03.06.2013	13:00:00	10	2 - 14			
19	02.07.2013	10:40:00	90	2 - 14			
20	13.08.2013	10:40:00	300	2 - 14			
21	03.09.2013	11:00:00	50	2 - 14			
22	24.09.2013	11:25:00	100	2 - 14			
23	05.11.2013	13:10:00	50	2 - 14			
24	03.12.2013	14:40:00	370	2 - 14			
25	14.01.2014	11:45:00	<DL				
26	11.02.2014	12:35:00	250	2 - 14			
27	11.03.2014	13:00:00	<DL				
28	08.04.2014	11:15:00	200	2 - 14			
29	06.05.2014	12:10:00	10	2 - 14			
30	10.06.2014	12:50:00	550	2 - 14			
31	08.07.2014	14:10:00	130	1, 2 - 14			
32	05.08.2014	13:05:00	<DL				
33	02.09.2014	10:55:00	100	2 - 14			
34	07.10.2014	11:40:00	450	2 - 14			
35	04.11.2014	11:35:00	80	2 - 14			
36	02.12.2014	13:20:00	170	2 - 14			
37	13.01.2015	12:35:00	100	2 - 14			
38	10.02.2015	12:10:00	40	1, 2 - 14			
39	10.03.2015	12:10:00	<DL				
40	07.04.2015	11:00:00	30	2 - 14			
41	05.05.2015	13:30:00	15	2 - 14			
42	02.06.2015	11:30:00	45	2 - 14			
43	07.07.2015	12:15:00	2000	2 - 14			
44	04.08.2015	10:55:00	3000	1, 2 - 14			
45	16.09.2015	11:05:00	6650	2 - 14			
46	06.10.2015	15:30:00	<DL	2 - 14			
47	17.11.2015	09:00:00	<DL				

Legionella Sample #	Sampling Date	Sampling Time	*Legionella* [cfu/100 mL]	Serology	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	HPC Sample #
48	01.12.2015	12:20:00	<DL				
49	12.01.2016	12:40:00	10	2 - 14			
50	02.02.2016	12:15:00	350	1, 2 - 14			
51	07.03.2016	13:20:00	100	1			
52	06.04.2016	13:30:00	130	L. sp.			
53	11.05.2016	10:14:00	90	2 - 14			
54	07.06.2016	11:02:00	175	1			
55	04.07.2016	10:31:00	1360	1			
56	02.08.2016	12:30:00	2100	1			
57	06.09.2016	13:04:00	400	1			
58	04.10.2016	11:46:00	450	1			
59	02.11.2016	11:53:00	12100	1, 2 - 14			
60	06.12.2016	11:04:00	250	1, 2 - 14			
61	03.01.2017	12:53:00	25	2 - 14	41	4040	1
62	07.02.2017	13:26:00	1380	2 - 14	270	1530	2
63	07.03.2017	11:31:00	750	2 - 14	97	315	3
64	04.04.2017	12:24:00	155	2 - 14	2160	37800	4
65	02.05.2017	10:43:00	5	2 - 14	82	176	5
66	06.06.2017	11:30:00	2250	2 - 14			
67	03.07.2017	11:13:00	1450	2 - 14	86400	64800	6
68	01.08.2017	11:00:00	2300	1, 2 - 14	48600	48600	7
69	05.09.2017	13:48:00	1800	1, 2 - 14	10800	2300	8
70	04.10.2017	11:29:00	17000	2 - 14	1080	1080	9
71	07.11.2017	12:07:00	1600	2 - 14	2500	1300	10
72	05.12.2017	14:08:00	1100	1	60	190	11
73	09.01.2018	13:19:00	2200	2 - 14	80	100	12
74	06.02.2018	11:41:00	280	1, 2 - 14	40	110	13
75	06.03.2018	12:52:00	140	2 - 14	30	10	14
76	10.04.2018	11:53:00	1200	2 - 14	1060	1090	15
77	08.05.2018	10:52:00	800	2 - 14	3000	960	16
78	05.06.2018	13:18:00	3900	2 - 14	27000	16200	17
79	03.07.2018	12:50:00	<DL		2060	2800	18
80	07.08.2018	12:00:00	3000	2 - 14	3900	1590	19
81	04.09.2018	10:10:00	1100	1, 2 - 14	420	43200	20
82	01.10.2018	12:30:00	1200	2 - 14	1100	1700	21
83	05.11.2018	08:26:00	360	1, 2 - 14	2300	500000	22
84	03.12.2018	10:11:00	490	1	470	1590	23
	07.01.2019	10:29	300		240	140	24
	04.02.2019	12:17	380		120	2000	25
	05.03.2019	09:39	1100		470	250	26
	01.04.2019	12:45	700		500000	500000	27
	06.05.2019	12:35	400		940	1700	28
	03.06.2019	12:25	560		670	600	29
	01.07.2019	12:04	15		500	500000	30
	05.08.2019	11:25	4300		5200	2900	31
	02.09.2019	12:06	22000		320	840	32
	07.10.2019	10:56	1800		760	5400	33
	04.11.2019	10:27	1300		250	2100	34

Legionella Sample #	Sampling Date	Sampling Time	*Legionella* [cfu/100 mL]	Serology	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]	HPC Sample #
	02.12.2019	10:51	4500		580	720	35
	06.01.2020	12:54	1200		110	70	
	03.02.2020	12:50	150		90	50	
	02.03.2020	11:18	1500		80	120	
	06.04.2020	11:12	100		480	5800	
	04.05.2020	11:04	930		500	330	
	03.08.2020	10:44	400		890	500000	
	07.09.2020	11:35	800		190	220	
	06.01.2020	10:29	75		110	70	
	03.02.2020	12:17	1100		90	50	

DA Table B 41: Cooling Tower 4 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 5 days.

Sample #	Biocide dosages 5 days before sampling	Biocide dosages 5 days after sampling	Biocide dosages 5 days before and after sampling	Ratio of dosages 5 days after and before sampling	Mean mV 5 days before sampling	Time [h] below threshold 5 days before sampling
1	30	29	59	0.966666667	384.6960833	68
2	46	39	85	0.847826087	396.6940833	71
3	51	47	98	0.921568627	430.7794167	49
4	21	8	29	0.380952381	393.18125	86
5	38	46	84	1.210526316	418.9249167	48
6	52	53	105	1.019230769	442.14625	42
7	54	48	102	0.888888889	408.9038333	56
8	55	56	111	1.018181818	420.62825	53
9	49	48	97	0.979591837	431.60775	51
10	42	46	88	1.095238095	437.8826667	45
11	37	46	83	1.243243243	417.00925	55
12	35	40	75	1.142857143	401.29075	58
13	35	54	89	1.542857143	446.9139402	44
14	10	15	25	1.5	594.1281285	1
15	44	45	89	1.022727273	500.9975759	7
16	41	41	82	1	497.9444526	13
17	58	54	112	0.931034483	496.4721797	3
18	46	39	85	0.847826087	493.3879736	18
19	38	41	79	1.078947368	498.4551018	9
20	36	49	85	1.361111111	481.2127881	18
21	33	44	77	1.333333333	484.0948361	5
22	16	17	33	1.0625	494.7401632	0
23	20	23	43	1.15	503.061201	0
24	25	21	46	0.84	488.7826619	0
25	27	47	74	1.740740741	431.3726992	58
26	43	32	75	0.744186047	524.5934219	13
27	50	44	94	0.88	487.190242	23
28	43	53	96	1.23255814	458.8873006	31
29	44	36	80	0.818181818	500.0966131	26
30	47	35	82	0.744680851	491.7524396	21
31	50	46	96	0.92	480.7410311	26
32	38	35	73	0.921052632	461.8182988	36
33	47	55	102	1.170212766	455.2204638	34
34	44	32	76	0.727272727	456.0885376	23
35	31	36	67	1.161290323	459.1325127	20
36	51	52	103	1.019607843	476.8829259	12
37	41	54	95	1.317073171	477.2045855	11
38	52	53	105	1.019230769	474.3222315	7
39	41	39	80	0.951219512	453.6426633	17
40	36	35	71	0.972222222	416.3399577	35
41	28	17	45	0.607142857	476.5783211	15
42	46	37	83	0.804347826	450.8632632	44
43	42	48	90	1.142857143	474.620949	7
44	43	40	83	0.930232558	463.2550084	1
45	25	29	54	1.16	465.9845144	0
46	36	37	73	1.027777778	455.1280375	1
47	46	54	100	1.173913043	487.0705651	29

Sample #	Biocide dosages 5 days before sampling	Biocide dosages 5 days after sampling	Biocide dosages 5 days before and after sampling	Ratio of dosages 5 days after and before sampling	Mean mV 5 days before sampling	Time [h] below threshold 5 days before sampling
48	33	21	54	0.636363636	457.094372	43
49	16	17	33	1.0625	402.7457466	32
50	43	26	69	0.604651163	425.6568029	22
51	18	18	36	1	406.5148158	22
52	38	44	82	1.157894737	414.4213816	34
53	35	32	67	0.914285714	412.892333	39
54	41	32	73	0.780487805	433.2112905	27
55	28	32	60	1.142857143	390.7692973	71
56	27	26	53	0.962962963	413.6991175	33
57	44	49	93	1.113636364	434.7785128	25
58	29	34	63	1.172413793	430.4627853	28
59	48	39	87	0.8125	433.6478372	24
60	38	38	76	1	448.3083471	20
61	33	32	65	0.96969697	420.6434731	26
62	40	36	76	0.9	433.8187927	27
63	47	36	83	0.765957447	427.0319529	31
64	8	30	38	3.75	376.6542946	104
65	28	23	51	0.821428571	369.6538534	73
66	32	28	60	0.875	440.6294245	18
67	21	23	44	1.095238095	429.0597473	3
68	31	31	62	1	448.5817433	12
69	15	17	32	1.133333333	438.4727183	0
70	44	26	70	0.590909091	430.2067108	21
71	0	0	0	0	424.9400113	0
72	0	8	8	8	449.7711057	0
73	18	15	33	0.833333333	461.5784714	0
74	20	24	44	1.2	459.7626129	0
75	19	20	39	1.052631579	458.5499481	0
76	20	23	43	1.15	443.475941	17
77	20	31	51	1.55	440.7169683	6
78	37	37	74	1	437.595828	12
79	28	22	50	0.785714286	443.3366943	14
80	24	21	45	0.875	445.6954718	9
81	38	28	66	0.736842105	445.5445346	13
82	16	21	37	1.3125	444.1038155	9
83	22	16	38	0.727272727	464.2042648	3
84	19	18	37	0.947368421	483.4131241	0

DA Table B 42: Cooling Tower 4 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 5 days.

Sample #	Biocide dosages 5 days before sampling	Biocide dosages 5 days after sampling	Biocide dosages 5 days before and after sampling	Ratio of dosages 5 days after and before sampling	Mean mV 5 days before sampling	Time [h] below threshold 5 days before sampling
1	33	32	65	0.96969697	420.6434731	26
2	40	36	76	0.9	433.8187927	27
3	47	36	83	0.765957447	427.0319529	31
4	8	30	38	3.75	376.6542946	104
5	28	23	51	0.821428571	369.6538534	73
6	21	23	44	1.095238095	429.0597473	3
7	31	31	62	1	448.5817433	12
8	15	17	32	1.133333333	438.4727183	0
9	44	26	70	0.590909091	430.2067108	21
10	0	0	0	0	424.9400113	0
11	0	8	8	8	449.7711057	0
12	18	15	33	0.833333333	461.5784714	0
13	20	24	44	1.2	459.7626129	0
14	19	20	39	1.052631579	458.5499481	0
15	20	23	43	1.15	443.475941	17
16	20	31	51	1.55	440.7169683	6
17	37	37	74	1	437.595828	12
18	28	22	50	0.785714286	443.3366943	14
19	24	21	45	0.875	445.6954718	9
20	38	28	66	0.736842105	445.5445346	13
21	16	21	37	1.3125	444.1038155	9
22	22	16	38	0.727272727	464.2042648	3
23	19	18	37	0.947368421	483.4131241	0
24	26	23	49	0.884615385	460.5637563	5
25	21	19	40	0.904761905	445.7468269	2
26	28	22	50	0.785714286	441.8390976	5
27	25	32	57	1.28	446.6995742	5
28	19	17	36	0.894736842	461.7780843	1
29	24	21	45	0.875	458.3156253	4
30	18	16	34	0.888888889	487.9657379	8
31	26	39	65	1.5	438.9644852	11
32	21	30	51	1.428571429	444.0561353	2
33	19	18	37	0.947368421	463.9789668	2
34	20	24	44	1.2	453.8005737	5
35	25	23	48	0.92	453.9419184	4

DA Table B 43: Cooling Tower 4 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 7 days.

Sample #	Biocide dosages 7 days before sampling	Biocide dosages 7 days after sampling	Biocide dosages 7 days before and after sampling	Ratio of dosages 7 days after and before sampling	Mean mV 7 days before sampling	Time [h] below threshold 7 days before sampling
1	43	50	93	1.162790698	386.65625	95
2	65	60	125	0.923076923	396.9825	96
3	71	65	136	0.915492958	423.5545833	73
4	30	17	47	0.566666667	394.7495833	119
5	46	66	112	1.434782609	414.05	72
6	75	74	149	0.986666667	441.58125	61
7	74	69	143	0.932432432	412.30375	77
8	76	78	154	1.026315789	418.8858333	75
9	71	72	143	1.014084507	435.3304167	71
10	60	62	122	1.033333333	434.245	65
11	55	64	119	1.163636364	419.91625	73
12	50	56	106	1.12	407.3958333	76
13	50	71	121	1.42	446.0396374	64
14	26	29	55	1.115384615	541.2857527	18
15	62	60	122	0.967741935	502.2022545	8
16	55	58	113	1.054545455	495.6193696	16
17	78	71	149	0.91025641	491.749543	6
18	69	56	125	0.811594203	489.1040114	27
19	52	51	103	0.980769231	498.16027	18
20	47	66	113	1.404255319	477.816603	20
21	46	63	109	1.369565217	483.9363237	6
22	23	19	42	0.826086957	501.7499737	0
23	30	33	63	1.1	500.468796	1
24	37	31	68	0.837837838	491.3139816	1
25	40	67	107	1.675	419.1881205	87
26	62	42	104	0.677419355	523.1129538	21
27	71	62	133	0.873239437	486.4074813	29
28	59	77	136	1.305084746	455.9568209	42
29	65	50	115	0.769230769	493.482371	38
30	69	47	116	0.68115942	487.1572331	33
31	73	64	137	0.876712329	482.7249685	32
32	54	53	107	0.981481481	467.7882778	47
33	64	67	131	1.046875	444.811672	54
34	66	41	107	0.621212121	453.0867876	32
35	49	48	97	0.979591837	460.2202054	27
36	70	75	145	1.071428571	475.8094468	16
37	56	77	133	1.375	476.9790898	14
38	71	73	144	1.028169014	473.5175887	11
39	57	59	116	1.035087719	469.0536423	17
40	56	57	113	1.017857143	438.5697499	45
41	37	17	54	0.459459459	475.1408888	18
42	65	49	114	0.753846154	468.6785841	57
43	63	64	127	1.015873016	468.5208551	7
44	59	59	118	1	463.5438258	1
45	33	44	77	1.333333333	464.4053294	0
46	44	49	93	1.113636364	447.7772146	11
47	58	76	134	1.310344828	482.7830794	44

Sample #	Biocide dosages 7 days before sampling	Biocide dosages 7 days after sampling	Biocide dosages 7 days before and after sampling	Ratio of dosages 7 days after and before sampling	Mean mV 7 days before sampling	Time [h] below threshold 7 days before sampling
48	54	43	97	0.796296296	462.7278953	59
49	19	25	44	1.315789474	405.9838925	32
50	63	43	106	0.682539683	425.9590414	32
51	28	23	51	0.821428571	401.8297628	36
52	40	55	95	1.375	414.452575	36
53	45	44	89	0.977777778	414.3061701	50
54	58	49	107	0.844827586	438.0193193	32
55	40	51	91	1.275	402.4469081	81
56	38	45	83	1.184210526	410.4225651	59
57	61	59	120	0.967213115	434.2405901	34
58	42	48	90	1.142857143	428.5710486	40
59	67	57	124	0.850746269	436.35671	32
60	54	52	106	0.962962963	447.387001	28
61	44	46	90	1.045454545	420.7388765	38
62	58	48	106	0.827586207	435.2535591	34
63	61	55	116	0.901639344	424.1948609	38
64	17	44	61	2.588235294	379.7646991	139
65	46	32	78	0.695652174	384.4929357	95
66	45	43	88	0.955555556	442.3843682	26
67	32	37	69	1.15625	433.0737735	7
68	44	48	92	1.090909091	448.0978626	16
69	20	32	52	1.6	433.1589966	0
70	61	44	105	0.721311475	427.7943847	26
71	0	0	0	0	424.0558181	0
72	0	15	15	15	448.8869126	0
73	25	15	40	0.6	463.9093028	0
74	28	31	59	1.107142857	463.0226824	0
75	27	27	54	1	462.8814327	0
76	28	33	61	1.178571429	443.4586098	18
77	29	46	75	1.586206897	441.1828786	9
78	54	50	104	0.925925926	438.846887	18
79	38	33	71	0.868421053	440.066553	24
80	34	30	64	0.882352941	447.2598239	15
81	44	43	87	0.977272727	427.5455078	35
82	22	29	51	1.318181818	444.2228759	9
83	30	25	55	0.833333333	464.3892597	3
84	27	25	52	0.925925926	482.8377237	2

DA Table B 44: Cooling Tower 4 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 7 days.

Sample #	Biocide dosages 7 days before sampling	Biocide dosages 7 days after sampling	Biocide dosages 7 days before and after sampling	Ratio of dosages 7 days after and before sampling	Mean mV 7 days before sampling	Time [h] below threshold 7 days before sampling
1	44	46	90	1.045454545	420.7388765	38
2	58	48	106	0.827586207	435.2535591	34
3	61	55	116	0.901639344	424.1948609	38
4	17	44	61	2.588235294	379.7646991	139
5	46	32	78	0.695652174	384.4929357	95
6	32	37	69	1.15625	433.0737735	7
7	44	48	92	1.090909091	448.0978626	16
8	20	32	52	1.6	433.1589966	0
9	61	44	105	0.721311475	427.7943847	26
10	0	0	0	0	424.0558181	0
11	0	15	15	15	448.8869126	0
12	25	15	40	0.6	463.9093028	0
13	28	31	59	1.107142857	463.0226824	0
14	27	27	54	1	462.8814327	0
15	28	33	61	1.178571429	443.4586098	18
16	29	46	75	1.586206897	441.1828786	9
17	54	50	104	0.925925926	438.846887	18
18	38	33	71	0.868421053	440.066553	24
19	34	30	64	0.882352941	447.2598239	15
20	44	43	87	0.977272727	427.5455078	35
21	22	29	51	1.318181818	444.2228759	9
22	30	25	55	0.833333333	464.3892597	3
23	27	25	52	0.925925926	482.8377237	2
24	34	30	64	0.882352941	461.024975	5
25	29	26	55	0.896551724	445.4178321	4
26	36	30	66	0.833333333	442.3931592	11
27	31	46	77	1.483870968	445.0764886	8
28	27	26	53	0.962962963	459.1138208	1
29	34	32	66	0.941176471	459.0705368	4
30	30	16	46	0.533333333	473.8743691	22
31	36	54	90	1.5	441.0838836	15
32	33	41	74	1.242424242	444.6419087	3
33	28	24	52	0.857142857	458.7752028	2
34	27	34	61	1.259259259	452.427205	5
35	37	31	68	0.837837838	451.5421046	7

DA Table B 45: Cooling Tower 4 - Redox potential data for correlation analyses with *Legionella* data; redox potential-threshold: 400 mV, observation period: 10 days.

Sample #	Biocide dosages 10 days before sampling	Biocide dosages 10 days after sampling	Biocide dosages 10 days before and after sampling	Ratio of dosages 10 days after and before sampling	Mean mV 10 days before sampling	Time [h] below threshold 10 days before sampling
1	58	83	141	1.431034483	386.206652	134
2	93	89	182	0.956989247	403.3507917	132
3	101	99	200	0.98019802	420.6180417	108
4	51	41	92	0.803921569	399.0717573	164
5	62	98	160	1.580645161	409.6157917	110
6	107	99	206	0.925233645	439.8356667	91
7	106	100	206	0.943396226	420.357875	104
8	106	110	216	1.037735849	415.9744167	107
9	103	101	204	0.980582524	437.5729167	98
10	90	85	175	0.944444444	434.275625	92
11	82	90	172	1.097560976	420.4647303	104
12	75	83	158	1.106666667	411.4107083	105
13	72	99	171	1.375	441.7792389	95
14	53	58	111	1.094339623	507.7749147	46
15	91	86	177	0.945054945	497.7619971	9
16	76	80	156	1.052631579	501.1795207	20
17	107	96	203	0.897196262	492.0768848	9
18	102	85	187	0.833333333	489.1294321	44
19	75	78	153	1.04	499.765192	28
20	66	90	156	1.363636364	474.3547942	28
21	69	87	156	1.260869565	484.7784618	11
22	44	19	63	0.431818182	502.8405815	7
23	41	47	88	1.146341463	500.881711	1
24	49	45	94	0.918367347	485.7892843	18
25	54	96	150	1.777777778	432.9173003	100
26	92	69	161	0.75	520.0162847	35
27	104	87	191	0.836538462	491.8450028	40
28	84	108	192	1.285714286	458.4664645	62
29	95	73	168	0.768421053	491.2171926	56
30	100	73	173	0.73	489.8020063	42
31	104	89	193	0.855769231	485.9445412	45
32	75	84	159	1.12	469.3927231	64
33	91	77	168	0.846153846	456.9091423	67
34	96	56	152	0.583333333	459.3793683	36
35	72	69	141	0.958333333	459.8961374	41
36	91	108	199	1.186813187	471.616658	25
37	78	107	185	1.371794872	473.7841161	23
38	100	93	193	0.93	472.3179562	15
39	75	91	166	1.213333333	456.9360206	48
40	86	90	176	1.046511628	452.4912988	47
41	59	19	78	0.322033898	456.3631613	46
42	90	62	152	0.688888889	475.2819118	76
43	95	87	182	0.915789474	463.9960782	9
44	86	84	170	0.976744186	462.6307659	2
45	48	62	110	1.291666667	463.730571	0
46	55	60	115	1.090909091	438.7420808	35

Sample #	Biocide dosages 10 days before sampling	Biocide dosages 10 days after sampling	Biocide dosages 10 days before and after sampling	Ratio of dosages 10 days after and before sampling	Mean mV 10 days before sampling	Time [h] below threshold 10 days before sampling
47	77	105	182	1.363636364	471.9842832	59
48	86	71	157	0.825581395	471.3346213	78
49	23	38	61	1.652173913	411.0110034	32
50	83	52	135	0.626506024	419.6097342	67
51	37	46	83	1.243243243	399.3260141	68
52	47	67	114	1.425531915	416.0757099	37
53	64	75	139	1.171875	417.0635111	62
54	87	73	160	0.83908046	440.7515408	40
55	62	75	137	1.209677419	410.8362216	98
56	54	67	121	1.240740741	409.499556	80
57	89	67	156	0.752808989	433.2139866	46
58	63	63	126	1	427.8203574	59
59	93	81	174	0.870967742	439.556579	44
60	73	72	145	0.98630137	446.9040347	39
61	59	67	126	1.13559322	421.3983932	55
62	85	71	156	0.835294118	434.5796206	50
63	75	86	161	1.146666667	422.3931758	52
64	25	66	91	2.64	384.7341627	191
65	66	36	102	0.545454545	363.6466925	143
66	72	62	134	0.861111111	441.5155204	37
67	51	55	106	1.078431373	433.1232321	15
68	61	68	129	1.114754098	446.0398874	20
69	29	55	84	1.896551724	427.8122903	0
70	84	62	146	0.738095238	426.8428041	33
71	0	0	0	0	422.7111074	0
72	0	27	27	27	447.5606227	0
73	37	19	56	0.513513514	464.5059359	1
74	41	43	84	1.048780488	463.9738475	2
75	33	38	71	1.151515152	471.5955607	0
76	42	50	92	1.19047619	442.923387	21
77	46	65	111	1.413043478	440.3225922	13
78	79	68	147	0.860759494	439.0657166	22
79	51	52	103	1.019607843	440.0516942	30
80	53	47	100	0.886792453	447.6786722	23
81	57	62	119	1.087719298	433.6378911	43
82	33	39	72	1.181818182	443.5117106	10
83	44	36	80	0.818181818	463.9162897	4
84	42	37	79	0.880952381	483.881618	3

DA Table B 46: Cooling Tower 4 - Redox potential data for correlation analyses with HPC data; redox potential-threshold: 400 mV, observation period: 10 days.

Sample #	Biocide dosages 10 days before sampling	Biocide dosages 10 days after sampling	Biocide dosages 10 days before and after sampling	Ratio of dosages 10 days after and before sampling	Mean mV 10 days before sampling	Time [h] below threshold 10 days before sampling
1	59	67	126	1.13559322	421.3983932	55
2	85	71	156	0.835294118	434.5796206	50
3	75	86	161	1.146666667	422.3931758	52
4	25	66	91	2.64	384.7341627	191
5	66	36	102	0.545454545	363.6466925	143
6	51	55	106	1.078431373	433.1232321	15
7	61	68	129	1.114754098	446.0398874	20
8	29	55	84	1.896551724	427.8122903	0
9	84	62	146	0.738095238	426.8428041	33
10	0	0	0	0	422.7111074	0
11	0	27	27	27	447.5606227	0
12	37	19	56	0.513513514	464.5059359	1
13	41	43	84	1.048780488	463.9738475	2
14	33	38	71	1.151515152	471.5955607	0
15	42	50	92	1.19047619	442.923387	21
16	46	65	111	1.413043478	440.3225922	13
17	79	68	147	0.860759494	439.0657166	22
18	51	52	103	1.019607843	440.0516942	30
19	53	47	100	0.886792453	447.6786722	23
20	57	62	119	1.087719298	433.6378911	43
21	33	39	72	1.181818182	443.5117106	10
22	44	36	80	0.818181818	463.9162897	4
23	42	37	79	0.880952381	483.881618	3
24	47	42	89	0.893617021	461.6580727	5
25	42	36	78	0.857142857	445.8137084	5
26	51	41	92	0.803921569	441.9412674	14
27	45	62	107	1.377777778	446.6636745	13
28	40	40	80	1	454.9758827	1
29	53	53	106	1	455.4077501	5
30	51	17	68	0.333333333	469.3134087	28
31	56	72	128	1.285714286	440.5961292	21
32	56	51	107	0.910714286	444.1721499	8
33	42	44	86	1.047619048	457.2828976	7
34	39	48	87	1.230769231	452.9218737	9
35	49	45	94	0.918367347	452.8417843	7

DA Table B 47: Cooling Tower 4 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 25 °C, observation period: 5 days.

Sample #	Mean water temp. [°C] 5 days before sampling	Min. water temp. [°C] 5 days before sampling	Max. water temp. [°C] 5 days before sampling	Time [h] above threshold 5 days before sampling
1	18.4924	16.94	19.67	0
2	18.2087	16.475	19.338	0
3	19.1633	17.422	20.145	0
4	18.8693	16.934	20.183	0
5	20.3764	18.524	23.041	0
6	20.8125	18.747	24.551	0
7	24.43695	16.337	26.85	36
8	24.8239	23.315	26.544	60
9	22.28105	19.602	24.032	0
10	19.7161	18.175	21.292	0
11	19.2956	17.09	20.985	0
12	19.29005	17.966	20.477	0
13	19.6769	18.51	21.252	0
14	17.50263158	14.299	19.649	0
15	23.88735	21.692	26.3235	18
16	23.096825	21.5755	24.6345	0
17	26.261775	24.1505	27.4775	108
18	25.8381	22.579	28.823	96
19	27.36945	24.453	29.24	114
20	26.6267	22.1735	29.0175	90
21	28.43695	25.9605	30.129	120
22	27.6623	25.473	29.734	120
23	23.904225	22.5735	25.3525	18
24	23.512425	22.259	24.718	0
25	20.05665	17.901	21.493	0
26	23.6553	22.361	24.512	0
27	23.597475	21.578	25.1645	6
28	25.812375	23.181	27.4065	102
29	23.299225	21.757	25.9005	12
30	17.945	17.945	17.945	0
31	28.908725	26.4105	30.902	132
32	28.85785	27.6405	29.855	120
33	27.483525	24.437	29.401	114
34	21.5175	21.5175	21.5175	0
35	26.1376	23.6175	27.518	102
36	22.77835	20.7365	24.2335	0
37	23.50855	22.266	25.5325	6
38	22.7475	22.7475	22.7475	0
39	22.85265	21.0735	24.7565	0
40	22.3082	20.92	24.35	0
41	24.27069444	15.3125	28.247	42
42	26.23175	25.0195	28.835	120
43	31.401575	28.2195	34.0925	120
44	28.02755	24.5455	32.0495	114
45	27.7459	25.5125	29.6075	120
46	25.664225	22.598	27.869	72

Sample #	Mean water temp. [°C] 5 days before sampling	Min. water temp. [°C] 5 days before sampling	Max. water temp. [°C] 5 days before sampling	Time [h] above threshold 5 days before sampling
47	23.50025	21.222	25.7285	18
48	22.690875	20.8925	24.329	0
49	18.96	16.43	22.2	0
50	23.2324	20.7445	25.6715	12
51	22.461	21.2625	23.5355	0
52	24.28285	21.6685	27.0015	30
53	28.036225	26.228	29.285	120
54	29.9256	28.317	30.8855	120
55	27.89455	24.7195	29.6875	114
56	28.0661	25.676	29.9285	120
57	28.976125	26.56	30.603	120
58	28.707175	26.3425	30.536	120
59	26.1345	24.2295	28.0325	102
60	23.0308	21.3485	25.1625	12
61	23.60012687	20.835	24.513	0
62	23.54052917	21.28	25.817	26
63	23.75904167	21.516	25.8175	12
64	25.77506667	22.2705	27.8945	87
65	23.9971375	21.529	25.9525	28
66	28.8452625	25.66	31.5895	120
67	29.01867083	25.328	31.001	120
68	27.83586555	25.629	29.8985	119
69	17.65972083	14.895	20.3365	0
70	32.9050431	26.8365	36	120
71	26.16275417	26.0465	26.2795	120
72	24.84407917	24.7275	24.9605	0
73	24.1686875	22.402	25.5975	15
74	23.71989167	21.338	25.435	9
75	24.89412917	22.4815	27.8935	52
76	25.99365	23.3125	28.4275	84
77	25.79076667	23.3395	28.6925	83
78	29.94668333	27.7525	32.443	120
79	28.20734167	26.573	30.4685	120
80	30.07016807	27.0475	32.4315	119
81	28.62410417	25.9765	31.489	120
82	26.05074583	23.448	28.0395	103
83	23.63004583	21.8835	25.394	6
84	21.5925	21.5925	21.5925	0

DA Table B 48: Cooling Tower 4 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 25 °C, observation period: 5 days.

Sample #	Mean water temp. [°C] 5 days before sampling	Min. water temp. [°C] 5 days before sampling	Max. water temp. [°C] 5 days before sampling	Time [h] above threshold 5 days before sampling
1	23.60012687	20.835	24.513	0
2	23.54052917	21.28	25.817	26
3	23.75904167	21.516	25.8175	12
4	25.77506667	22.2705	27.8945	87
5	23.9971375	21.529	25.9525	28
6	29.01867083	25.328	31.001	120
7	27.83586555	25.629	29.8985	119
8	17.65972083	14.895	20.3365	0
9	32.9050431	26.8365	36	120
10	26.16275417	26.0465	26.2795	120
11	24.84407917	24.7275	24.9605	0
12	24.1686875	22.402	25.5975	15
13	23.71989167	21.338	25.435	9
14	24.89412917	22.4815	27.8935	52
15	25.99365	23.3125	28.4275	84
16	25.79076667	23.3395	28.6925	83
17	29.94668333	27.7525	32.443	120
18	28.20734167	26.573	30.4685	120
19	30.07016807	27.0475	32.4315	119
20	28.62410417	25.9765	31.489	120
21	26.05074583	23.448	28.0395	103
22	23.63004583	21.8835	25.394	6
23	21.5925	21.5925	21.5925	0

DA Table B 49: Cooling Tower 4 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 25 °C, observation period: 7 days.

Sample #	Mean water temp. [°C] 7 days before sampling	Min. water temp. [°C] 7 days before sampling	Max. water temp. [°C] 7 days before sampling	Time [h] above threshold 7 days before sampling
1	18.67260714	16.94	20.869	0
2	18.20764286	16.475	19.338	0
3	19.45260714	17.422	20.83	0
4	19.20964286	16.934	21.653	0
5	21.17310714	18.524	24.912	0
6	21.36282143	18.747	24.624	0
7	24.56653571	16.337	26.85	60
8	24.79753571	22.839	26.544	84
9	22.86939286	19.602	25.374	18
10	20.26082143	18.175	24.082	0
11	19.36217857	17.09	20.985	0
12	19.25	17.8	20.477	0
13	19.60571429	18.251	21.252	0
14	18.14022222	14.299	20.912	0
15	24.02748214	21.692	26.3235	24
16	23.29903571	21.5755	24.6345	0
17	25.63478571	22.1125	27.4775	108
18	26.05164286	22.579	28.823	144
19	27.04735714	24.453	29.24	156
20	25.84825	21.295	29.087	102
21	28.32628571	25.9605	30.129	168
22	27.24319643	25.473	29.734	168
23	23.78339286	22.4695	25.3525	18
24	23.67864286	22.259	25.4435	6
25	21.16596429	17.901	24.623	0
26	23.6772037	22.361	24.512	0
27	23.52648214	21.578	25.1645	6
28	25.56907143	23.037	27.4065	132
29	24.09294643	21.757	27.2545	60
30	17.945	17.945	17.945	0
31	26.37863462	17.945	30.902	132
32	28.83866071	26.7685	31.013	168
33	27.19985714	24.437	29.401	156
34	21.5175	21.5175	21.5175	0
35	25.56323214	22.395	27.518	114
36	22.89464286	20.7365	24.2335	0
37	23.36366071	20.8805	25.5325	6
38	22.7475	22.7475	22.7475	0
39	22.88980357	21.0735	24.7565	0
40	22.39469643	20.92	24.35	0
41	24.273	15.3125	28.247	54
42	25.53758929	21.773	28.835	132
43	31.116625	28.2195	34.0925	168
44	27.76692857	24.5455	32.0495	162
45	27.54739286	25.5125	29.6075	168
46	25.40066071	22.1585	27.869	96

Sample #	Mean water temp. [°C] 7 days before sampling	Min. water temp. [°C] 7 days before sampling	Max. water temp. [°C] 7 days before sampling	Time [h] above threshold 7 days before sampling
47	23.591625	21.222	25.7285	18
48	22.74003571	20.8925	24.329	0
49	20.02046429	16.43	24.218	0
50	23.33344643	20.7445	25.6715	18
51	22.48114286	21.2625	23.54	0
52	23.86069643	21.6685	27.0015	30
53	27.27932143	23.2525	29.285	150
54	29.751625	28.25	31.096	168
55	28.08042857	24.7195	29.6875	162
56	28.29705357	25.676	29.9285	168
57	29.00685714	26.56	30.7905	168
58	28.65517857	26.3425	30.536	168
59	26.08184483	24.2295	28.0325	132
60	23.08591071	21.3485	25.1625	12
61	23.50552	20.835	24.513	0
62	23.37160417	21.28	25.817	28
63	23.70470238	21.516	25.8175	12
64	25.88107143	22.2705	27.8945	135
65	23.66449702	20.996	25.9525	28
66	28.62640179	25.396	31.5895	168
67	28.84413393	25.328	31.001	168
68	28.41432635	25.629	30.6105	167
69	18.20710714	14.895	21.4795	0
70	31.83392683	26.167	36	168
71	26.20970536	26.0465	26.373	168
72	24.89103274	24.7275	25.054	28
73	24.08375	22.402	25.5975	17
74	23.67482738	21.338	26.562	11
75	25.01649107	22.4815	27.8935	81
76	26.01767857	23.3125	28.4275	131
77	25.6111994	23.3065	28.6925	113
78	30.28133631	27.7525	32.443	168
79	28.15281548	26.473	30.4685	168
80	29.96497605	27.0475	32.4315	167
81	28.89153274	25.9765	31.489	168
82	25.74801786	22.8575	28.0395	130
83	23.7869375	21.8835	25.4025	11
84	21.93466964	21.1125	24.465	0

DA Table B 50: Cooling Tower 4 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 25 °C, observation period: 7 days.

Sample #	Mean water temp. [°C] 7 days before sampling	Min. water temp. [°C] 7 days before sampling	Max. water temp. [°C] 7 days before sampling	Time [h] above threshold 7 days before sampling
1	23.50552	20.835	24.513	0
2	23.37160417	21.28	25.817	28
3	23.70470238	21.516	25.8175	12
4	25.88107143	22.2705	27.8945	135
5	23.66449702	20.996	25.9525	28
6	28.84413393	25.328	31.001	168
7	28.41432635	25.629	30.6105	167
8	18.20710714	14.895	21.4795	0
9	31.83392683	26.167	36	168
10	26.20970536	26.0465	26.373	168
11	24.89103274	24.7275	25.054	28
12	24.08375	22.402	25.5975	17
13	23.67482738	21.338	26.562	11
14	25.01649107	22.4815	27.8935	81
15	26.01767857	23.3125	28.4275	131
16	25.6111994	23.3065	28.6925	113
17	30.28133631	27.7525	32.443	168
18	28.15281548	26.473	30.4685	168
19	29.96497605	27.0475	32.4315	167
20	28.89153274	25.9765	31.489	168
21	25.74801786	22.8575	28.0395	130
22	23.7869375	21.8835	25.4025	11
23	21.93466964	21.1125	24.465	0

DA Table B 51: Cooling Tower 4 - Water temperature data for correlation analyses with *Legionella* data; water temperature-threshold: 25 °C, observation period: 10 days.

Sample #	Mean water temp. [°C] 10 days before sampling	Min. water temp. [°C] 10 days before sampling	Max. water temp. [°C] 10 days before sampling	Time [h] above threshold 10 days before sampling
1	19.17265789	16.94	22.525	0
2	18.2327	16.475	19.808	0
3	19.346625	17.422	20.83	0
4	19.339475	16.666	21.719	0
5	21.594525	18.524	24.912	0
6	22.102725	18.747	28.234	18
7	24.195775	16.337	26.85	72
8	24.500525	22.122	26.544	102
9	23.154275	19.602	25.374	18
10	20.0926	17.975	24.082	0
11	19.14380488	16.57	20.985	0
12	19.43965	17.8	21.895	0
13	19.67755	18.251	21.252	0
14	18.47820513	14.299	21.177	0
15	23.9160125	21.692	26.3235	24
16	23.2497	21.5755	24.6345	0
17	25.0825875	21.9385	27.5155	114
18	25.7198125	22.579	28.823	186
19	27.320875	24.453	29.24	228
20	25.4798625	20.8765	32.2085	114
21	28.4982625	25.9605	30.404	240
22	27.240775	25.1145	30.0435	240
23	24.32605	22.4695	27.665	65
24	23.947075	22.259	26.0075	18
25	22.018125	17.901	25.4855	18
26	23.48098718	21.985	24.659	0
27	23.27795	21.578	25.1645	6
28	25.17701282	23.037	27.4065	144
29	24.5576375	21.757	27.2545	120
30	17.945	17.945	17.945	0
31	23.71538158	17.945	30.902	132
32	29.181925	26.7685	31.2335	240
33	26.65275641	15.824	29.401	222
34	21.5175	21.5175	21.5175	0
35	25.40869512	22.395	27.518	168
36	23.0841125	20.7365	25.3935	12
37	23.0964625	20.8805	25.5325	6
38	22.4666	12.2175	23.36	0
39	22.95348718	21.0735	25.5345	6
40	22.59705128	20.92	24.35	0
41	24.25510526	15.3125	28.247	84
42	24.236775	19.2925	28.835	132
43	30.344825	25.774	34.0925	240
44	27.816425	24.5455	32.0495	234
45	27.51655	25.5125	29.6075	240
46	25.415725	22.1585	27.869	144

Sample #	Mean water temp. [°C] 10 days before sampling	Min. water temp. [°C] 10 days before sampling	Max. water temp. [°C] 10 days before sampling	Time [h] above threshold 10 days before sampling
47	24.1869375	21.222	27.8305	60
48	22.5727	20.8925	24.329	0
49	20.64415	16.43	24.218	0
50	23.6087625	20.7445	25.962	36
51	22.4269	21.2625	23.54	0
52	23.8833875	21.6685	27.0015	30
53	26.5331	21.784	29.285	186
54	29.6295125	28.25	31.413	240
55	28.4109125	24.7195	32.2545	234
56	29.053775	25.676	32.08	240
57	29.50085	26.56	33.8125	240
58	28.5786375	25.2315	30.792	240
59	25.53442683	22.584	28.0325	132
60	23.0880875	21.3485	25.1625	12
61	23.67372414	20.835	26.605	30
62	23.32682292	21.28	25.817	28
63	23.82193333	21.516	25.8175	38
64	25.3848159	21.2435	27.8945	157
65	23.70415625	20.996	25.9525	41
66	29.17680417	25.396	32.828	240
67	29.0413375	25.328	31.001	240
68	28.73034937	25.629	31.7035	239
69	18.2975375	14.895	21.4795	0
70	30.35183051	25.8225	36	240
71	26.28112448	26.0465	26.516	240
72	24.96146875	24.7275	25.195	100
73	24.09710369	22.402	26.322	52
74	23.13601875	19.677	26.562	11
75	24.73752917	22.4815	27.8935	86
76	25.59940833	23.0445	28.4275	154
77	25.93985833	23.3065	29.5315	173
78	30.29839167	27.7525	32.443	240
79	27.93473958	26.191	30.4685	240
80	29.68800209	26.296	32.4315	239
81	28.60769583	25.151	31.489	240
82	25.94774792	22.8575	28.0395	202
83	24.00790041	21.8835	26.772	31
84	22.26045625	21.1125	25.184	2

DA Table B 52: Cooling Tower 4 - Water temperature data for correlation analyses with HPC data; water temperature-threshold: 25 °C, observation period: 10 days.

Sample #	Mean water temp. [°C] 10 days before sampling	Min. water temp. [°C] 10 days before sampling	Max. water temp. [°C] 10 days before sampling	Time [h] above threshold 10 days before sampling
1	23.67372414	20.835	26.605	30
2	23.32682292	21.28	25.817	28
3	23.82193333	21.516	25.8175	38
4	25.3848159	21.2435	27.8945	157
5	23.70415625	20.996	25.9525	41
6	29.0413375	25.328	31.001	240
7	28.73034937	25.629	31.7035	239
8	18.2975375	14.895	21.4795	0
9	30.35183051	25.8225	36	240
10	26.28112448	26.0465	26.516	240
11	24.96146875	24.7275	25.195	100
12	24.09710369	22.402	26.322	52
13	23.13601875	19.677	26.562	11
14	24.73752917	22.4815	27.8935	86
15	25.59940833	23.0445	28.4275	154
16	25.93985833	23.3065	29.5315	173
17	30.29839167	27.7525	32.443	240
18	27.93473958	26.191	30.4685	240
19	29.68800209	26.296	32.4315	239
20	28.60769583	25.151	31.489	240
21	25.94774792	22.8575	28.0395	202
22	24.00790041	21.8835	26.772	31
23	22.26045625	21.1125	25.184	2

DA Table B 53: Retrospective HPC data for the comparison of HPC at the incubation temperatures of 22 and 36 °C.

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1	42nd BImSchV scope	1180	740
2	42nd BImSchV scope	4320	194400
3	42nd BImSchV scope	16200	11400
4	42nd BImSchV scope	10800	6600
5	42nd BImSchV scope	81000	91800
6	42nd BImSchV scope	21600	16200
7	42nd BImSchV scope	570	680
8	42nd BImSchV scope	220	260
9	42nd BImSchV scope	1130	1800
10	42nd BImSchV scope	130	180
11	42nd BImSchV scope	2300	TNTC
12	42nd BImSchV scope	740	190
13	42nd BImSchV scope	280	100
14	42nd BImSchV scope	360	420
15	42nd BImSchV scope	270	270
16	42nd BImSchV scope	830	1420
17	42nd BImSchV scope	2400	320
18	42nd BImSchV scope	100	60
19	42nd BImSchV scope	630	720
20	42nd BImSchV scope	960	670
21	42nd BImSchV scope	90	160
22	42nd BImSchV scope	1270	59400
23	42nd BImSchV scope	1390	1740
24	42nd BImSchV scope	380	5400
25	42nd BImSchV scope	10800	27000
26	42nd BImSchV scope	560	1800
27	42nd BImSchV scope	320	2400
28	42nd BImSchV scope	740	2400
29	42nd BImSchV scope	1890	16200
30	42nd BImSchV scope	<DL	200
31	42nd BImSchV scope	<DL	10
32	42nd BImSchV scope	<DL	<DL
33	42nd BImSchV scope	<DL	<DL
34	42nd BImSchV scope	21600	16200
35	42nd BImSchV scope	43200	10800
36	42nd BImSchV scope	27000	91800
37	42nd BImSchV scope	320	70
38	42nd BImSchV scope	20	10
39	42nd BImSchV scope	2130	16200
40	other industrial site	1410	2400
41	42nd BImSchV scope	10	<DL
42	42nd BImSchV scope	140	5100
43	42nd BImSchV scope	3000	16200

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
44	42nd BImSchV scope	160	40
45	42nd BImSchV scope	6800	4800
46	42nd BImSchV scope	740	30
47	42nd BImSchV scope	20400	3100
48	42nd BImSchV scope	12000	2500
49	42nd BImSchV scope	<DL	<DL
50	42nd BImSchV scope	4100	5500
51	42nd BImSchV scope	<DL	<DL
52	42nd BImSchV scope	880	690
53	42nd BImSchV scope	420	8400
54	42nd BImSchV scope	<DL	20
55	42nd BImSchV scope	<DL	<DL
56	42nd BImSchV scope	550	na
57	42nd BImSchV scope	<DL	360
58	42nd BImSchV scope	<DL	240
59	42nd BImSchV scope	5600	4000
60	42nd BImSchV scope	91800	124200
61	42nd BImSchV scope	151200	151200
62	42nd BImSchV scope	2500	8300
63	42nd BImSchV scope	2200	12700
64	42nd BImSchV scope	27000	253800
65	42nd BImSchV scope	<DL	40
66	42nd BImSchV scope	40	<DL
67	42nd BImSchV scope	5300	3800
68	other industrial site	520	580
69	42nd BImSchV scope	1090	3000
70	42nd BImSchV scope	32400	32400
71	42nd BImSchV scope	100	4200
72	42nd BImSchV scope	324000	129600
73	42nd BImSchV scope	151200	32400
74	42nd BImSchV scope	3600	3000
75	42nd BImSchV scope	240	16200
76	42nd BImSchV scope	1420	10800
77	42nd BImSchV scope	80	1460
78	42nd BImSchV scope	16200	9600
79	42nd BImSchV scope	345600	156600
80	42nd BImSchV scope	10800	10800
81	42nd BImSchV scope	90	310
82	42nd BImSchV scope	150	120
83	42nd BImSchV scope	151200	70200
84	42nd BImSchV scope	<DL	2400
85	42nd BImSchV scope	<DL	<DL
86	42nd BImSchV scope	250	260
87	42nd BImSchV scope	16200	3000
88	42nd BImSchV scope	490	4200

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
89	other industrial site	670	620
90	42nd BImSchV scope	3300	6600
91	42nd BImSchV scope	950	590
92	42nd BImSchV scope	18100	16200
93	42nd BImSchV scope	5900	54000
94	42nd BImSchV scope	2300	960
95	42nd BImSchV scope	172800	145800
96	42nd BImSchV scope	30	110
97	42nd BImSchV scope	TNTC	TNTC
98	42nd BImSchV scope	570	360
99	42nd BImSchV scope	97200	15500
100	42nd BImSchV scope	5300	9800
101	42nd BImSchV scope	<DL	<DL
102	42nd BImSchV scope	102600	140400
103	42nd BImSchV scope	400	50
104	42nd BImSchV scope	<DL	10
105	42nd BImSchV scope	640	2900
106	42nd BImSchV scope	40	20
107	42nd BImSchV scope	20	20
108	42nd BImSchV scope	14600	64800
109	42nd BImSchV scope	30	690
110	42nd BImSchV scope	10	240
111	42nd BImSchV scope	<DL	<DL
112	42nd BImSchV scope	<DL	50
113	42nd BImSchV scope	<DL	<DL
114	42nd BImSchV scope	<DL	20
115	42nd BImSchV scope	190	360
116	42nd BImSchV scope	37800	64800
117	42nd BImSchV scope	270	230
118	42nd BImSchV scope	<DL	10
119	42nd BImSchV scope	50	8300
120	other industrial site	150	1110
121	42nd BImSchV scope	440	360
122	42nd BImSchV scope	4200	4800
123	42nd BImSchV scope	216000	<DL
124	42nd BImSchV scope	48600	48600
125	42nd BImSchV scope	16200	21600
126	42nd BImSchV scope	324000	145800
127	42nd BImSchV scope	1320	540
128	42nd BImSchV scope	1230	210
129	42nd BImSchV scope	170	260
130	42nd BImSchV scope	1080	1360
131	42nd BImSchV scope	360	690
132	42nd BImSchV scope	470	1590
133	42nd BImSchV scope	440	580

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
134	42nd BImSchV scope	1670	860
135	42nd BImSchV scope	960	5400
136	42nd BImSchV scope	30	80
137	42nd BImSchV scope	160	220
138	42nd BImSchV scope	420	<DL
139	42nd BImSchV scope	1180	980
140	42nd BImSchV scope	630	2400
141	42nd BImSchV scope	210	360
142	42nd BImSchV scope	20	480
143	42nd BImSchV scope	1110	10800
144	42nd BImSchV scope	380	260
145	42nd BImSchV scope	380	16200
146	42nd BImSchV scope	1120	5400
147	42nd BImSchV scope	720	630
148	42nd BImSchV scope	100	110
149	42nd BImSchV scope	730	160
150	42nd BImSchV scope	1190	490
151	42nd BImSchV scope	3000	3600
152	42nd BImSchV scope	1670	5400
153	42nd BImSchV scope	30	90
154	42nd BImSchV scope	620	1140
155	42nd BImSchV scope	20	90
156	42nd BImSchV scope	10	20
157	42nd BImSchV scope	420	650
158	42nd BImSchV scope	30	200
159	42nd BImSchV scope	1320	16200
160	42nd BImSchV scope	10	150
161	42nd BImSchV scope	<DL	<DL
162	42nd BImSchV scope	<DL	<DL
163	42nd BImSchV scope	<DL	<DL
164	42nd BImSchV scope	<DL	<DL
165	42nd BImSchV scope	48600	124200
166	42nd BImSchV scope	59400	118800
167	42nd BImSchV scope	270	210
168	42nd BImSchV scope	<DL	10
169	42nd BImSchV scope	2200	3000
170	42nd BImSchV scope	<DL	10
171	42nd BImSchV scope	20	<DL
172	42nd BImSchV scope	30	40
173	42nd BImSchV scope	50	10
174	42nd BImSchV scope	<DL	2160
175	42nd BImSchV scope	27000	21600
176	42nd BImSchV scope	300	5400
177	other industrial site	470	2700
178	42nd BImSchV scope	4800	1230

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
179	42nd BImSchV scope	14040	4800
180	42nd BImSchV scope	16200	8900
181	42nd BImSchV scope	64800	97200
182	42nd BImSchV scope	640	1630
183	42nd BImSchV scope	2160	31200
184	42nd BImSchV scope	2160	32400
185	42nd BImSchV scope	30	160
186	42nd BImSchV scope	1280	3000
187	42nd BImSchV scope	17400	9700
188	42nd BImSchV scope	10800	2300
189	42nd BImSchV scope	60	1080
190	42nd BImSchV scope	90	100
191	42nd BImSchV scope	10800	<DL
192	42nd BImSchV scope	2400	110
193	42nd BImSchV scope	500	30
194	42nd BImSchV scope	108000	18000
195	42nd BImSchV scope	490	4100
196	42nd BImSchV scope	<DL	10
197	42nd BImSchV scope	140	230
198	42nd BImSchV scope	12000	25800
199	42nd BImSchV scope	<DL	<DL
200	42nd BImSchV scope	<DL	<DL
201	42nd BImSchV scope	16200	19200
202	42nd BImSchV scope	10	40
203	42nd BImSchV scope	91800	124200
204	42nd BImSchV scope	39000	37200
205	42nd BImSchV scope	1310	970
206	42nd BImSchV scope	<DL	<DL
207	42nd BImSchV scope	240	1900
208	42nd BImSchV scope	5700	2800
209	42nd BImSchV scope	40	<DL
210	42nd BImSchV scope	760	189000
211	42nd BImSchV scope	40	180
212	42nd BImSchV scope	135000	129600
213	42nd BImSchV scope	150	20
214	42nd BImSchV scope	10	10
215	42nd BImSchV scope	102600	140400
216	42nd BImSchV scope	590	810
217	42nd BImSchV scope	90	<DL
218	42nd BImSchV scope	97200	124200
219	other industrial site	102600	1600
220	42nd BImSchV scope	380	660
221	42nd BImSchV scope	30	40
222	42nd BImSchV scope	18000	99000
223	42nd BImSchV scope	81000	97200

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
224	42nd BImSchV scope	39000	25200
225	42nd BImSchV scope	81000	70200
226	42nd BImSchV scope	5000	1920
227	42nd BImSchV scope	14600	10300
228	42nd BImSchV scope	1650	60
229	42nd BImSchV scope	120	130
230	42nd BImSchV scope	20	90
231	42nd BImSchV scope	30	40
232	42nd BImSchV scope	240	140
233	42nd BImSchV scope	190	890
234	42nd BImSchV scope	60	140
235	42nd BImSchV scope	40	20
236	42nd BImSchV scope	<DL	10
237	42nd BImSchV scope	60	350
238	42nd BImSchV scope	11800	10100
239	42nd BImSchV scope	210	80
240	42nd BImSchV scope	330	410
241	42nd BImSchV scope	80	40
242	42nd BImSchV scope	190	40
243	42nd BImSchV scope	1900	7900
244	42nd BImSchV scope	160	90
245	42nd BImSchV scope	120	7400
246	42nd BImSchV scope	390	1900
247	42nd BImSchV scope	320	40
248	42nd BImSchV scope	340	540
249	42nd BImSchV scope	6500	3300
250	42nd BImSchV scope	<DL	<DL
251	42nd BImSchV scope	<DL	<DL
252	42nd BImSchV scope	5800	870
253	42nd BImSchV scope	90	260
254	42nd BImSchV scope	<DL	<DL
255	42nd BImSchV scope	<DL	<DL
256	42nd BImSchV scope	<DL	60
257	42nd BImSchV scope	<DL	<DL
258	42nd BImSchV scope	<DL	<DL
259	42nd BImSchV scope	<DL	<DL<
260	42nd BImSchV scope	<DL	<DL
261	42nd BImSchV scope	<DL	<DL
262	42nd BImSchV scope	2700	760
263	42nd BImSchV scope	13200	6000
264	42nd BImSchV scope	16600	18000
265	42nd BImSchV scope	<DL	<DL
266	42nd BImSchV scope	140	140
267	42nd BImSchV scope	1830	5000
268	42nd BImSchV scope	230	12800

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
269	42nd BImSchV scope	<DL	172800
270	42nd BImSchV scope	10	<DL
271	42nd BImSchV scope	70	30
272	42nd BImSchV scope	<DL	<DL
273	42nd BImSchV scope	<DL	10
274	42nd BImSchV scope	<DL	180
275	42nd BImSchV scope	60	60
276	42nd BImSchV scope	81000	91800
277	42nd BImSchV scope	25800	24000
278	42nd BImSchV scope	102600	189000
279	42nd BImSchV scope	330	90
280	42nd BImSchV scope	160	810
281	42nd BImSchV scope	<DL	<DL
282	42nd BImSchV scope	10	<DL
283	42nd BImSchV scope	<DL	<DL
284	42nd BImSchV scope	80	260
285	42nd BImSchV scope	90	230
286	42nd BImSchV scope	110	240
287	42nd BImSchV scope	130	230
288	42nd BImSchV scope	170	310
289	42nd BImSchV scope	140	100
290	42nd BImSchV scope	180	320
291	42nd BImSchV scope	160	250
292	42nd BImSchV scope	80	220
293	42nd BImSchV scope	540000	540000
294	42nd BImSchV scope	7000	6300
295	42nd BImSchV scope	113400	TNTC
296	42nd BImSchV scope	TNTC	TNTC
297	42nd BImSchV scope	11400	31800
298	42nd BImSchV scope	TNTC	TNTC
299	other industrial site	91800	36000
300	42nd BImSchV scope	780	390
301	42nd BImSchV scope	460	130
302	42nd BImSchV scope	40	30
303	42nd BImSchV scope	27000	6200
304	42nd BImSchV scope	21600	6000
305	42nd BImSchV scope	27000	64800
306	42nd BImSchV scope	97200	91800
307	42nd BImSchV scope	2900	21600
308	42nd BImSchV scope	10	880
309	other industrial site	2100	4200
310	42nd BImSchV scope	130	12600
311	42nd BImSchV scope	162000	54000
312	42nd BImSchV scope	2800	6900
313	42nd BImSchV scope	20	10

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
314	42nd BImSchV scope	8900	910
315	42nd BImSchV scope	4600	10100
316	42nd BImSchV scope	<DL	9500
317	42nd BImSchV scope	<DL	<DL
318	42nd BImSchV scope	<DL	<DL
319	42nd BImSchV scope	<DL	<DL
320	42nd BImSchV scope	80	70
321	42nd BImSchV scope	363600	2600
322	42nd BImSchV scope	580	4500
323	42nd BImSchV scope	1130	5900
324	42nd BImSchV scope	<DL	<DL
325	42nd BImSchV scope	51000	64800
326	42nd BImSchV scope	9400	11300
327	42nd BImSchV scope	106200	102600
328	42nd BImSchV scope	140400	75600
329	other industrial site	1700	1100
330	42nd BImSchV scope	180	50
331	42nd BImSchV scope	10800	4400
332	42nd BImSchV scope	27000	na
333	42nd BImSchV scope	129600	TNTC
334	42nd BImSchV scope	10800	1010
335	42nd BImSchV scope	151200	81000
336	42nd BImSchV scope	<DL	<DL
337	42nd BImSchV scope	170	30
338	42nd BImSchV scope	1800	20
339	42nd BImSchV scope	4200	33000
340	42nd BImSchV scope	610	7800
341	other industrial site	2700	6000
342	42nd BImSchV scope	6700	3900
343	42nd BImSchV scope	7500	8600
344	42nd BImSchV scope	33000	18600
345	42nd BImSchV scope	440	580
346	42nd BImSchV scope	91800	35400
347	42nd BImSchV scope	10500	14200
348	42nd BImSchV scope	7400	na
349	42nd BImSchV scope	na	na
350	42nd BImSchV scope	na	na
351	42nd BImSchV scope	na	na
352	42nd BImSchV scope	120	2000
353	42nd BImSchV scope	na	na
354	42nd BImSchV scope	310	530
355	42nd BImSchV scope	20	10
356	42nd BImSchV scope	10	10
357	42nd BImSchV scope	1150	na
358	42nd BImSchV scope	2280	1350

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
359	42nd BImSchV scope	na	120
360	42nd BImSchV scope	na	570
361	42nd BImSchV scope	na	na
362	42nd BImSchV scope	na	130
363	42nd BImSchV scope	na	22200
364	42nd BImSchV scope	90	20
365	42nd BImSchV scope	na	5700
366	42nd BImSchV scope	1280	3500
367	42nd BImSchV scope	780	50
368	42nd BImSchV scope	20	<DL
369	42nd BImSchV scope	10	30
370	42nd BImSchV scope	1550	1260
371	42nd BImSchV scope	2900	na
372	42nd BImSchV scope	2050	3000
373	42nd BImSchV scope	70	na
374	42nd BImSchV scope	<DL	na
375	42nd BImSchV scope	<DL	na
376	42nd BImSchV scope	190	na
377	42nd BImSchV scope	<DL	na
378	42nd BImSchV scope	102600	243000
379	42nd BImSchV scope	145800	113400
380	42nd BImSchV scope	460	na
381	42nd BImSchV scope	60	na
382	42nd BImSchV scope	<DL	10
383	42nd BImSchV scope	<DL	<DL
384	42nd BImSchV scope	<DL	<DL
385	42nd BImSchV scope	480	150
386	42nd BImSchV scope	19500	3000
387	42nd BImSchV scope	140	130
388	42nd BImSchV scope	210	320
389	42nd BImSchV scope	40	1480
390	other industrial site	60	50
391	42nd BImSchV scope	1870	640
392	42nd BImSchV scope	32400	32400
393	42nd BImSchV scope	27000	21600
394	42nd BImSchV scope	180	2400
395	42nd BImSchV scope	TNTC	583200
396	other industrial site	180	370
397	42nd BImSchV scope	59400	10800
398	42nd BImSchV scope	167400	54000
399	42nd BImSchV scope	75600	TNTC
400	42nd BImSchV scope	610	16200
401	42nd BImSchV scope	<DL	130
402	42nd BImSchV scope	37800	27000
403	42nd BImSchV scope	<DL	<DL

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
404	42nd BImSchV scope	97200	108000
405	42nd BImSchV scope	2300	280
406	42nd BImSchV scope	<DL	<DL
407	42nd BImSchV scope	<DL	<DL
408	42nd BImSchV scope	130	7300
409	42nd BImSchV scope	64800	113400
410	42nd BImSchV scope	2400	6100
411	42nd BImSchV scope	102600	118800
412	42nd BImSchV scope	90	20
413	42nd BImSchV scope	1020	1900
414	42nd BImSchV scope	4800	64200
415	42nd BImSchV scope	113400	124200
416	42nd BImSchV scope	60	510
417	42nd BImSchV scope	27000	48600
418	42nd BImSchV scope	30000	21600
419	42nd BImSchV scope	108000	54000
420	42nd BImSchV scope	660	2500
421	42nd BImSchV scope	7100	21000
422	42nd BImSchV scope	4600	5400
423	42nd BImSchV scope	<DL	20
424	42nd BImSchV scope	102600	TNTC
425	42nd BImSchV scope	6500	370
426	42nd BImSchV scope	10	<DL
427	42nd BImSchV scope	20	10
428	42nd BImSchV scope	150	90
429	42nd BImSchV scope	<DL	120
430	42nd BImSchV scope	3400	37800
431	42nd BImSchV scope	330	3700
432	42nd BImSchV scope	1570	1200
433	42nd BImSchV scope	630	440
434	42nd BImSchV scope	1380	1330
435	42nd BImSchV scope	100	120
436	other industrial site	350	480
437	42nd BImSchV scope	<DL	<DL
438	42nd BImSchV scope	<DL	<DL
439	42nd BImSchV scope	330	280
440	42nd BImSchV scope	640	940
441	42nd BImSchV scope	16200	1900
442	42nd BImSchV scope	21600	7200
443	42nd BImSchV scope	<DL	<DL
444	42nd BImSchV scope	<DL	<DL
445	42nd BImSchV scope	<DL	20
446	42nd BImSchV scope	<DL	40
447	42nd BImSchV scope	21600	1540
448	42nd BImSchV scope	32400	10800

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
449	42nd BImSchV scope	340	5400
450	42nd BImSchV scope	16200	16200
451	42nd BImSchV scope	18000	27000
452	42nd BImSchV scope	11400	16200
453	42nd BImSchV scope	8400	21600
454	other industrial site	320	520
455	42nd BImSchV scope	4100	1500
456	42nd BImSchV scope	81000	4800
457	42nd BImSchV scope	1290	850
458	42nd BImSchV scope	2200	2300
459	42nd BImSchV scope	5100	1900
460	42nd BImSchV scope	18000	6700
461	42nd BImSchV scope	690	760
462	42nd BImSchV scope	710	80
463	42nd BImSchV scope	2200	2500
464	42nd BImSchV scope	80	<DL
465	42nd BImSchV scope	100	30
466	42nd BImSchV scope	380	100
467	42nd BImSchV scope	40	60
468	42nd BImSchV scope	470	250
469	42nd BImSchV scope	230	920
470	42nd BImSchV scope	70	<DL
471	42nd BImSchV scope	30	20
472	42nd BImSchV scope	150	200
473	42nd BImSchV scope	90	130
474	42nd BImSchV scope	130	100
475	42nd BImSchV scope	30	10
476	42nd BImSchV scope	130	200
477	42nd BImSchV scope	80	90
478	42nd BImSchV scope	<DL	<DL
479	42nd BImSchV scope	1400	2800
480	42nd BImSchV scope	160	130
481	42nd BImSchV scope	240	690
482	42nd BImSchV scope	150	<DL
483	42nd BImSchV scope	50	10
484	42nd BImSchV scope	270	50
485	42nd BImSchV scope	1600	2300
486	42nd BImSchV scope	10	60
487	42nd BImSchV scope	<DL	600
488	42nd BImSchV scope	<DL	8100
489	42nd BImSchV scope	<DL	37800
490	42nd BImSchV scope	<DL	59400
491	42nd BImSchV scope	<DL	2700
492	other industrial site	<DL	2100
493	42nd BImSchV scope	240	3500

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
494	42nd BImSchV scope	38000	TNTC
495	other industrial site	270	250
496	42nd BImSchV scope	2200	1640
497	42nd BImSchV scope	140	160
498	42nd BImSchV scope	20	60
499	42nd BImSchV scope	350	80
500	42nd BImSchV scope	<DL	<DL
501	42nd BImSchV scope	<DL	10
502	42nd BImSchV scope	10	110
503	42nd BImSchV scope	20	<DL
504	42nd BImSchV scope	670	470
505	42nd BImSchV scope	27000	7500
506	42nd BImSchV scope	1220	5200
507	42nd BImSchV scope	2700	3600
508	42nd BImSchV scope	180	15900
509	42nd BImSchV scope	<DL	10
510	42nd BImSchV scope	1800	6900
511	42nd BImSchV scope	<DL	10
512	42nd BImSchV scope	48600	81000
513	42nd BImSchV scope	2400	2700
514	42nd BImSchV scope	324000	183600
515	42nd BImSchV scope	32400	64800
516	42nd BImSchV scope	156600	91800
517	other industrial site	1760	4000
518	42nd BImSchV scope	5000	4100
519	42nd BImSchV scope	150	600
520	42nd BImSchV scope	18000	2700
521	42nd BImSchV scope	1900	4500
522	42nd BImSchV scope	324000	540000
523	42nd BImSchV scope	590	1120
524	42nd BImSchV scope	135000	12300
525	42nd BImSchV scope	11500	21000
526	42nd BImSchV scope	70200	70200
527	42nd BImSchV scope	1800	1800
528	42nd BImSchV scope	2400	70200
529	42nd BImSchV scope	1200	18600
530	other industrial site	390	900
531	42nd BImSchV scope	2580	2230
532	42nd BImSchV scope	110	210
533	42nd BImSchV scope	180	50
534	42nd BImSchV scope	90	50
535	42nd BImSchV scope	170	110
536	42nd BImSchV scope	16200	167400

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
537	42nd BImSchV scope	10	<DL
538	42nd BImSchV scope	<DL<	<DL
539	42nd BImSchV scope	<DL	<DL
540	42nd BImSchV scope	<DL	<DL
541	42nd BImSchV scope	10	<DL
542	42nd BImSchV scope	70200	580
543	42nd BImSchV scope	2400	790
544	42nd BImSchV scope	32400	10800
545	42nd BImSchV scope	1700	2120
546	42nd BImSchV scope	<DL	<DL
547	42nd BImSchV scope	97200	345600
548	42nd BImSchV scope	520	5400
549	42nd BImSchV scope	30	50
550	42nd BImSchV scope	360	200
551	42nd BImSchV scope	10	60
552	42nd BImSchV scope	48600	151200
553	42nd BImSchV scope	<DL	<DL
554	42nd BImSchV scope	<DL	<DL
555	42nd BImSchV scope	<DL	10
556	42nd BImSchV scope	10	40
557	42nd BImSchV scope	30	60
558	42nd BImSchV scope	40	380
559	42nd BImSchV scope	30	80
560	42nd BImSchV scope	3600	860
561	42nd BImSchV scope	16200	16200
562	42nd BImSchV scope	<DL	<DL
563	42nd BImSchV scope	110	10800
564	42nd BImSchV scope	243000	561600
565	42nd BImSchV scope	345600	496800
566	42nd BImSchV scope	2100	130
567	42nd BImSchV scope	486000	TNTC
568	42nd BImSchV scope	TNTC	410400
569	other industrial site	130	80
570	42nd BImSchV scope	4600	7100
571	42nd BImSchV scope	23400	24600
572	42nd BImSchV scope	4300	2600
573	42nd BImSchV scope	8100	10200
574	42nd BImSchV scope	10	10
575	42nd BImSchV scope	910	620
576	42nd BImSchV scope	80	10
577	42nd BImSchV scope	170	160
578	42nd BImSchV scope	60	30
579	42nd BImSchV scope	90	10
580	42nd BImSchV scope	TNTC	TNTC
581	42nd BImSchV scope	2	1

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
582	42nd BImSchV scope	10	50
583	42nd BImSchV scope	110	10
584	42nd BImSchV scope	30	30
585	42nd BImSchV scope	210	20
586	42nd BImSchV scope	80	410
587	42nd BImSchV scope	350	30
588	42nd BImSchV scope	100	800
589	42nd BImSchV scope	50	60
590	42nd BImSchV scope	10	10
591	42nd BImSchV scope	930	2200
592	42nd BImSchV scope	220	20
593	42nd BImSchV scope	420	410
594	42nd BImSchV scope	5500	14400
595	42nd BImSchV scope	80	20
596	42nd BImSchV scope	260	240
597	42nd BImSchV scope	4100	2400
598	42nd BImSchV scope	<DL	3900
599	42nd BImSchV scope	40	40
600	42nd BImSchV scope	1400	2800
601	42nd BImSchV scope	160	110
602	42nd BImSchV scope	<DL	<DL
603	42nd BImSchV scope	<DL	<DL
604	42nd BImSchV scope	<DL	<DL
605	42nd BImSchV scope	<DL	<DL
606	42nd BImSchV scope	<DL	<DL
607	other industrial site	<DL	<DL
608	42nd BImSchV scope	18000	4600
609	42nd BImSchV scope	33000	36000
610	42nd BImSchV scope	160	270
611	42nd BImSchV scope	60	8200
612	42nd BImSchV scope	16200	33000
613	other industrial site	30	760
614	42nd BImSchV scope	91800	24000
615	42nd BImSchV scope	TNTC	25800
616	42nd BImSchV scope	TNTC	37800
617	42nd BImSchV scope	4700	1800
618	42nd BImSchV scope	3800	1700
619	42nd BImSchV scope	1080	5400
620	42nd BImSchV scope	40	70
621	42nd BImSchV scope	108000	21600
622	42nd BImSchV scope	48600	16200
623	42nd BImSchV scope	30	10
624	42nd BImSchV scope	<DL	<DL
625	42nd BImSchV scope	2900	2200
626	42nd BImSchV scope	80	40

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
627	42nd BImSchV scope	100	60
628	42nd BImSchV scope	140	240
629	42nd BImSchV scope	90	20
630	42nd BImSchV scope	260	160
631	42nd BImSchV scope	200	400
632	42nd BImSchV scope	190	180
633	42nd BImSchV scope	80	100
634	42nd BImSchV scope	50	70
635	42nd BImSchV scope	TNTC	TNTC
636	42nd BImSchV scope	15200	160
637	42nd BImSchV scope	680	380
638	42nd BImSchV scope	20400	7600
639	42nd BImSchV scope	38400	21000
640	42nd BImSchV scope	60	2000
641	42nd BImSchV scope	2600	370
642	42nd BImSchV scope	13800	21800
643	42nd BImSchV scope	28200	33000
644	42nd BImSchV scope	13800	590
645	42nd BImSchV scope	2900	330
646	42nd BImSchV scope	324000	124200
647	42nd BImSchV scope	330	500
648	42nd BImSchV scope	90	130
649	42nd BImSchV scope	11200	70200
650	42nd BImSchV scope	189000	189000
651	42nd BImSchV scope	390	7100
652	42nd BImSchV scope	6200	2300
653	42nd BImSchV scope	<DL	<DL
654	42nd BImSchV scope	<DL	<DL
655	42nd BImSchV scope	20	<DL
656	42nd BImSchV scope	<DL	<DL
657	42nd BImSchV scope	163800	151200
658	42nd BImSchV scope	74400	91800
659	42nd BImSchV scope	27100	18000
660	42nd BImSchV scope	<DL	10
661	42nd BImSchV scope	<DL	<DL
662	42nd BImSchV scope	350	220
663	42nd BImSchV scope	370	500
664	42nd BImSchV scope	<DL	<DL
665	42nd BImSchV scope	<DL	<DL
666	42nd BImSchV scope	20	20
667	42nd BImSchV scope	12800	7500
668	42nd BImSchV scope	10	<DL
669	42nd BImSchV scope	80400	102600
670	42nd BImSchV scope	6300	370
671	42nd BImSchV scope	770	220

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
672	42nd BImSchV scope	<DL	<DL
673	42nd BImSchV scope	27000	22800
674	42nd BImSchV scope	19800	9000
675	42nd BImSchV scope	75600	59400
676	42nd BImSchV scope	48600	37800
677	42nd BImSchV scope	10800	10800
678	42nd BImSchV scope	30	10
679	42nd BImSchV scope	680	540
680	42nd BImSchV scope	<DL	20
681	42nd BImSchV scope	380	720
682	42nd BImSchV scope	302400	2400
683	42nd BImSchV scope	81000	145800
684	42nd BImSchV scope	450	1460
685	42nd BImSchV scope	1590	1300
686	42nd BImSchV scope	20	20
687	42nd BImSchV scope	43200	113400
688	42nd BImSchV scope	37800	4200
689	42nd BImSchV scope	170	3000
690	42nd BImSchV scope	302400	TNTC
691	42nd BImSchV scope	324000	TNTC
692	42nd BImSchV scope	367200	TNTC
693	other industrial site	140	720
694	42nd BImSchV scope	5700	7200
695	42nd BImSchV scope	5000	10800
696	42nd BImSchV scope	1800	900
697	42nd BImSchV scope	3800	2400
698	42nd BImSchV scope	15000	21600
699	42nd BImSchV scope	1080	640
700	42nd BImSchV scope	150	140
701	42nd BImSchV scope	110	110
702	42nd BImSchV scope	3300	1670
703	42nd BImSchV scope	15000	91800
704	42nd BImSchV scope	145800	10200
705	42nd BImSchV scope	97200	620
706	42nd BImSchV scope	124200	780
707	42nd BImSchV scope	480	5400
708	42nd BImSchV scope	<DL	<DL
709	42nd BImSchV scope	<DL	<DL
710	42nd BImSchV scope	<DL	<DL
711	42nd BImSchV scope	<DL	<DL
712	other industrial site	190	1420
713	42nd BImSchV scope	<DL	<DL
714	42nd BImSchV scope	10800	10800
715	42nd BImSchV scope	16200	16200
716	42nd BImSchV scope	21600	27000

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
717	42nd BImSchV scope	32400	75600
718	42nd BImSchV scope	10800	43200
719	42nd BImSchV scope	3600	4800
720	42nd BImSchV scope	80	40
721	42nd BImSchV scope	780	860
722	42nd BImSchV scope	340	150
723	42nd BImSchV scope	200	40
724	42nd BImSchV scope	940	1700
725	42nd BImSchV scope	180	360
726	42nd BImSchV scope	180	140
727	42nd BImSchV scope	160	520
728	42nd BImSchV scope	360	4800
729	42nd BImSchV scope	190	140
730	42nd BImSchV scope	440	190
731	42nd BImSchV scope	840	280
732	42nd BImSchV scope	710	310
733	42nd BImSchV scope	310	420
734	42nd BImSchV scope	3600	2100
735	42nd BImSchV scope	920	2500
736	42nd BImSchV scope	180	200
737	42nd BImSchV scope	320	960
738	42nd BImSchV scope	1020	2400
739	42nd BImSchV scope	180	90
740	42nd BImSchV scope	800	720
741	42nd BImSchV scope	860	590
742	42nd BImSchV scope	220	16200
743	42nd BImSchV scope	TNTC	75600
744	42nd BImSchV scope	720	370
745	42nd BImSchV scope	16200	1730
746	42nd BImSchV scope	70200	81000
747	42nd BImSchV scope	21600	10800
748	42nd BImSchV scope	440	320
749	42nd BImSchV scope	10	390
750	42nd BImSchV scope	40	50
751	42nd BImSchV scope	<DL	<DL
752	42nd BImSchV scope	<DL	<DL
753	42nd BImSchV scope	<DL	<DL
754	42nd BImSchV scope	<DL	<DL
755	42nd BImSchV scope	600	1900
756	42nd BImSchV scope	30	130
757	42nd BImSchV scope	<DL	<DL
758	42nd BImSchV scope	<DL	<DL
759	42nd BImSchV scope	<DL	10
760	42nd BImSchV scope	<DL	<DL
761	42nd BImSchV scope	<DL	<DL

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
762	42nd BImSchV scope	<DL	<DL
763	other industrial site	<DL	<DL
764	42nd BImSchV scope	39600	21600
765	42nd BImSchV scope	97200	32400
766	42nd BImSchV scope	TNTC	TNTC
767	42nd BImSchV scope	TNTC	TNTC
768	42nd BImSchV scope	59400	70200
769	42nd BImSchV scope	2250	780
770	42nd BImSchV scope	100	170
771	42nd BImSchV scope	8900	1050
772	42nd BImSchV scope	2040	960
773	42nd BImSchV scope	3000	1500
774	42nd BImSchV scope	3300	1490
775	42nd BImSchV scope	24600	7400
776	42nd BImSchV scope	8100	6000
777	42nd BImSchV scope	2400	5700
778	42nd BImSchV scope	1360	2400
779	42nd BImSchV scope	190	20
780	42nd BImSchV scope	190	5600
781	42nd BImSchV scope	9100	8800
782	42nd BImSchV scope	1680	5000
783	42nd BImSchV scope	20	3300
784	42nd BImSchV scope	6300	1030
785	42nd BImSchV scope	42000	59400
786	42nd BImSchV scope	380	430
787	42nd BImSchV scope	10400	11700
788	42nd BImSchV scope	<DL	<DL
789	42nd BImSchV scope	151200	162000
790	other industrial site	70	880
791	42nd BImSchV scope	<DL	50
792	42nd BImSchV scope	1170	3300
793	42nd BImSchV scope	194400	118800
794	42nd BImSchV scope	54000	102600
795	42nd BImSchV scope	43200	59400
796	42nd BImSchV scope	1800	5400
797	42nd BImSchV scope	102600	113400
798	42nd BImSchV scope	680	4800
799	42nd BImSchV scope	16200	151200
800	42nd BImSchV scope	530	470
801	42nd BImSchV scope	1670	5400
802	42nd BImSchV scope	1080	205200
803	42nd BImSchV scope	570	1500
804	42nd BImSchV scope	2400	3200
805	42nd BImSchV scope	172800	11800
806	42nd BImSchV scope	870	770

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
807	42nd BImSchV scope	820	680
808	42nd BImSchV scope	2400	30780
809	42nd BImSchV scope	40	210
810	42nd BImSchV scope	10	130
811	42nd BImSchV scope	32400	91800
812	42nd BImSchV scope	16200	70200
813	42nd BImSchV scope	183600	518400
814	42nd BImSchV scope	2200	2300
815	42nd BImSchV scope	100	16200
816	42nd BImSchV scope	3640	16200
817	42nd BImSchV scope	4800	5400
818	42nd BImSchV scope	110	160
819	42nd BImSchV scope	59400	27000
820	42nd BImSchV scope	21600	16200
821	42nd BImSchV scope	600	290
822	42nd BImSchV scope	4400	6600
823	42nd BImSchV scope	3900	2000
824	42nd BImSchV scope	18600	390
825	42nd BImSchV scope	<DL	<DL
826	42nd BImSchV scope	57600	24000
827	42nd BImSchV scope	9600	6600
828	42nd BImSchV scope	1740	9600
829	42nd BImSchV scope	10500	7800
830	42nd BImSchV scope	5800	5000
831	42nd BImSchV scope	<DL	<DL
832	42nd BImSchV scope	<DL	<DL
833	42nd BImSchV scope	7200	2600
834	42nd BImSchV scope	3800	1600
835	42nd BImSchV scope	<DL	110
836	42nd BImSchV scope	180	660
837	42nd BImSchV scope	<DL	<DL
838	42nd BImSchV scope	<DL	<DL
839	42nd BImSchV scope	<DL	10
840	42nd BImSchV scope	<DL	<DL
841	42nd BImSchV scope	310	660
842	42nd BImSchV scope	20	450
843	42nd BImSchV scope	790	320
844	42nd BImSchV scope	380	180
845	42nd BImSchV scope	660	290
846	42nd BImSchV scope	650	180
847	42nd BImSchV scope	20	20
848	42nd BImSchV scope	TNTC	183600
849	42nd BImSchV scope	329400	191400
850	42nd BImSchV scope	TNTC	226800
851	42nd BImSchV scope	<DL<DL	0

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
852	42nd BImSchV scope	110	560
853	42nd BImSchV scope	360	310
854	42nd BImSchV scope	<DL	<DL
855	42nd BImSchV scope	<DL	<DL
856	42nd BImSchV scope	<DL	<DL
857	42nd BImSchV scope	8900	7800
858	42nd BImSchV scope	75600	22800
859	42nd BImSchV scope	<DL	<DL
860	42nd BImSchV scope	8300	7900
861	42nd BImSchV scope	26400	25200
862	42nd BImSchV scope	2000	2000
863	42nd BImSchV scope	6100	21600
864	42nd BImSchV scope	20	50
865	42nd BImSchV scope	38	66
866	42nd BImSchV scope	<DL	70
867	42nd BImSchV scope	220	330
868	42nd BImSchV scope	830	480
869	42nd BImSchV scope	240	290
870	42nd BImSchV scope	670	600
871	42nd BImSchV scope	420	3900
872	42nd BImSchV scope	100	170
873	42nd BImSchV scope	330	280
874	42nd BImSchV scope	40	10
875	42nd BImSchV scope	250	200
876	42nd BImSchV scope	480	500
877	42nd BImSchV scope	200	150
878	42nd BImSchV scope	30	40
879	42nd BImSchV scope	280	730
880	42nd BImSchV scope	360	570
881	42nd BImSchV scope	1010	1800
882	42nd BImSchV scope	300	140
883	42nd BImSchV scope	210	300
884	42nd BImSchV scope	490	1500
885	42nd BImSchV scope	2900	18900
886	42nd BImSchV scope	220	150
887	42nd BImSchV scope	30	120
888	42nd BImSchV scope	170	200
889	42nd BImSchV scope	<DL	<DL
890	42nd BImSchV scope	50	<DL
891	42nd BImSchV scope	<DL	<DL
892	42nd BImSchV scope	620	570
893	42nd BImSchV scope	270	5100
894	42nd BImSchV scope	6900	5400
895	42nd BImSchV scope	620	250
896	42nd BImSchV scope	<DL	10

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
897	42nd BImSchV scope	<DL	<DL
898	42nd BImSchV scope	<DL	10
899	42nd BImSchV scope	80	<DL
900	42nd BImSchV scope	<DL	<DL
901	42nd BImSchV scope	<DL	<DL
902	42nd BImSchV scope	<DL	<DL
903	other industrial site	<DL	<DL
904	42nd BImSchV scope	32400	30600
905	42nd BImSchV scope	1980	3100
906	42nd BImSchV scope	1150	1070
907	other industrial site	2400	760
908	42nd BImSchV scope	21600	4200
909	42nd BImSchV scope	243000	81000
910	42nd BImSchV scope	162000	75600
911	42nd BImSchV scope	<DL	<DL
912	42nd BImSchV scope	2100	2900
913	42nd BImSchV scope	90	900
914	42nd BImSchV scope	1370	1800
915	42nd BImSchV scope	12000	10200
916	42nd BImSchV scope	850	7000
917	42nd BImSchV scope	90	12200
918	42nd BImSchV scope	140	230
919	42nd BImSchV scope	5400	59400
920	42nd BImSchV scope	180	90
921	42nd BImSchV scope	23400	42600
922	42nd BImSchV scope	1900	2300
923	42nd BImSchV scope	1800	13700
924	42nd BImSchV scope	1900	12700
925	42nd BImSchV scope	830	15600
926	42nd BImSchV scope	<DL	210
927	42nd BImSchV scope	<DL	40
928	42nd BImSchV scope	<DL	<DL
929	42nd BImSchV scope	3300	320
930	42nd BImSchV scope	51600	35400
931	42nd BImSchV scope	40	<DL
932	42nd BImSchV scope	3200	2900
933	42nd BImSchV scope	17400	3300
934	42nd BImSchV scope	40800	64800
935	42nd BImSchV scope	1600	1900
936	42nd BImSchV scope	9100	5100
937	42nd BImSchV scope	7800	13800
938	42nd BImSchV scope	40800	920
939	42nd BImSchV scope	50400	81000
940	42nd BImSchV scope	46800	2500
941	42nd BImSchV scope	59400	28200

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
942	42nd BImSchV scope	28800	39000
943	42nd BImSchV scope	172800	TNTC
944	42nd BImSchV scope	1280	1700
945	42nd BImSchV scope	64800	48600
946	42nd BImSchV scope	<DL	400
947	42nd BImSchV scope	5300	10800
948	other industrial site	13800	7000
949	42nd BImSchV scope	710	220
950	42nd BImSchV scope	10800	12000
951	42nd BImSchV scope	390	400
952	42nd BImSchV scope	530	480
953	42nd BImSchV scope	440	1100
954	42nd BImSchV scope	900	1600
955	42nd BImSchV scope	1200	710
956	42nd BImSchV scope	1220	1190
957	42nd BImSchV scope	39000	64800
958	42nd BImSchV scope	24600	28200
959	42nd BImSchV scope	57000	70200
960	42nd BImSchV scope	259200	52800
961	42nd BImSchV scope	31800	24600
962	42nd BImSchV scope	9000	10800
963	42nd BImSchV scope	226800	221400
964	42nd BImSchV scope	81000	52800
965	42nd BImSchV scope	21000	13500
966	42nd BImSchV scope	20	<DL
967	42nd BImSchV scope	<DL	<DL
968	42nd BImSchV scope	205200	297000
969	42nd BImSchV scope	172800	189000
970	42nd BImSchV scope	1600	1700
971	42nd BImSchV scope	<DL	<DL
972	42nd BImSchV scope	460	2600
973	42nd BImSchV scope	1070	97200
974	42nd BImSchV scope	710	170
975	42nd BImSchV scope	<DL	<DL
976	42nd BImSchV scope	510	500
977	42nd BImSchV scope	1390	860
978	42nd BImSchV scope	<DL	<DL
979	42nd BImSchV scope	<DL	<DL
980	42nd BImSchV scope	<DL	<DL
981	42nd BImSchV scope	10	320
982	42nd BImSchV scope	160	40
983	42nd BImSchV scope	60	680
984	42nd BImSchV scope	170	350
985	42nd BImSchV scope	350	54000
986	42nd BImSchV scope	124200	151200

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
987	42nd BImSchV scope	460	240
988	42nd BImSchV scope	194400	151200
989	42nd BImSchV scope	151200	140400
990	42nd BImSchV scope	90	50
991	42nd BImSchV scope	120	20
992	42nd BImSchV scope	<DL	20
993	42nd BImSchV scope	410	20
994	42nd BImSchV scope	240	TNTC
995	42nd BImSchV scope	500	TNTC
996	42nd BImSchV scope	310	1400
997	42nd BImSchV scope	<DL	<DL
998	42nd BImSchV scope	170	200
999	42nd BImSchV scope	<DL	<DL
1000	42nd BImSchV scope	760	TNTC
1001	42nd BImSchV scope	300	210
1002	42nd BImSchV scope	120	10
1003	42nd BImSchV scope	70	80
1004	42nd BImSchV scope	460	180
1005	42nd BImSchV scope	310	390
1006	42nd BImSchV scope	230	280
1007	42nd BImSchV scope	270	150
1008	42nd BImSchV scope	1700	960
1009	42nd BImSchV scope	70	290
1010	42nd BImSchV scope	250	90
1011	42nd BImSchV scope	14000	32400
1012	42nd BImSchV scope	6500	32400
1013	42nd BImSchV scope	1020	440
1014	42nd BImSchV scope	170	50
1015	42nd BImSchV scope	<DL	10
1016	42nd BImSchV scope	20	40
1017	42nd BImSchV scope	2300	540
1018	42nd BImSchV scope	440	1100
1019	42nd BImSchV scope	340	300
1020	other industrial site	4700	5400
1021	other industrial site	1020	6000
1022	other industrial site	1000	4800
1023	other industrial site	4600	21600
1024	other industrial site	10	80
1025	other industrial site	80	360
1026	other industrial site	<DL	<DL
1027	other industrial site	<DL	<DL
1028	other industrial site	<DL	<DL
1029	other industrial site	<DL	<DL
1030	other industrial site	10	<DL
1031	other industrial site	<DL	<DL

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1032	42nd BImSchV scope	40	20
1033	42nd BImSchV scope	97200	124200
1034	42nd BImSchV scope	3200	10800
1035	other industrial site	4900	10800
1036	42nd BImSchV scope	19200	86400
1037	42nd BImSchV scope	120	110
1038	42nd BImSchV scope	540	5500
1039	42nd BImSchV scope	910	2800
1040	42nd BImSchV scope	1300	5000
1041	42nd BImSchV scope	43200	2300
1042	42nd BImSchV scope	10	<DL
1043	42nd BImSchV scope	40	8700
1044	42nd BImSchV scope	<DL	<DL
1045	42nd BImSchV scope	<DL	<DL
1046	42nd BImSchV scope	10	230
1047	42nd BImSchV scope	9600	25200
1048	42nd BImSchV scope	1900	6100
1049	other industrial site	3000	1370
1050	42nd BImSchV scope	1380	320
1051	42nd BImSchV scope	151200	91800
1052	42nd BImSchV scope	550	1160
1053	42nd BImSchV scope	23400	86400
1054	42nd BImSchV scope	1900	360
1055	42nd BImSchV scope	151200	172800
1056	42nd BImSchV scope	226800	388800
1057	42nd BImSchV scope	19800	59400
1058	42nd BImSchV scope	59400	27000
1059	42nd BImSchV scope	2700	1300
1060	42nd BImSchV scope	43200	102600
1061	42nd BImSchV scope	12600	320
1062	42nd BImSchV scope	620	59400
1063	42nd BImSchV scope	3400	10800
1064	42nd BImSchV scope	37200	91800
1065	42nd BImSchV scope	29400	32400
1066	42nd BImSchV scope	12000	16200
1067	42nd BImSchV scope	530	32400
1068	42nd BImSchV scope	19200	10800
1069	42nd BImSchV scope	90	120
1070	42nd BImSchV scope	4100	16200
1071	42nd BImSchV scope	<DL	50
1072	42nd BImSchV scope	930	1520
1073	42nd BImSchV scope	320	180
1074	42nd BImSchV scope	129600	97200
1075	42nd BImSchV scope	40	<DL
1076	42nd BImSchV scope	30	<DL

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1077	42nd BImSchV scope	680	1160
1078	42nd BImSchV scope	100	260
1079	42nd BImSchV scope	97200	TNTC
1080	42nd BImSchV scope	10800	2500
1081	42nd BImSchV scope	32400	520
1082	42nd BImSchV scope	5400	310
1083	other industrial site	5400	10800
1084	other industrial site	30	60
1085	other industrial site	<DL	<DL
1086	other industrial site	<DL	<DL
1087	other industrial site	580	7800
1088	other industrial site	<DL	<DL
1089	other industrial site	20	220
1090	other industrial site	30	160
1091	other industrial site	10	<DL
1092	other industrial site	75400	64800
1093	other industrial site	<DL	<DL
1094	42nd BImSchV scope	2500	21600
1095	other industrial site	3600	16200
1096	42nd BImSchV scope	4700	1800
1097	42nd BImSchV scope	750	680
1098	42nd BImSchV scope	10500	5200
1099	42nd BImSchV scope	17400	10500
1100	42nd BImSchV scope	<DL	<DL
1101	42nd BImSchV scope	750	820
1102	42nd BImSchV scope	3200	1460
1103	42nd BImSchV scope	730	680
1104	42nd BImSchV scope	4400	2200
1105	42nd BImSchV scope	2200	1030
1106	42nd BImSchV scope	<DL	<DL
1107	42nd BImSchV scope	3100	5100
1108	42nd BImSchV scope	TNTC	TNTC
1109	42nd BImSchV scope	TNTC	TNTC
1110	42nd BImSchV scope	TNTC	TNTC
1111	other industrial site	4100	4100
1112	42nd BImSchV scope	10800	54000
1113	42nd BImSchV scope	75600	102600
1114	42nd BImSchV scope	2100	540
1115	42nd BImSchV scope	1370	800
1116	42nd BImSchV scope	75600	91800
1117	42nd BImSchV scope	<DL	10
1118	42nd BImSchV scope	340	720
1119	42nd BImSchV scope	2400	3600
1120	42nd BImSchV scope	930	70200
1121	42nd BImSchV scope	3100	4800

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1122	42nd BImSchV scope	205200	151200
1123	42nd BImSchV scope	3060	9100
1124	other industrial site	1460	2400
1125	42nd BImSchV scope	6600	13500
1126	42nd BImSchV scope	1500	940
1127	42nd BImSchV scope	70200	59400
1128	42nd BImSchV scope	5200	2900
1129	42nd BImSchV scope	16800	145800
1130	42nd BImSchV scope	2800	4700
1131	42nd BImSchV scope	130	80
1132	42nd BImSchV scope	1120	1800
1133	42nd BImSchV scope	1140	na
1134	42nd BImSchV scope	5500	3100
1135	42nd BImSchV scope	5100	1800
1136	42nd BImSchV scope	1320	1020
1137	42nd BImSchV scope	1400	1400
1138	42nd BImSchV scope	7200	6000
1139	42nd BImSchV scope	7200	4900
1140	42nd BImSchV scope	3400	750
1141	42nd BImSchV scope	990	830
1142	42nd BImSchV scope	21600	14400
1143	42nd BImSchV scope	TNTC	TNTC
1144	42nd BImSchV scope	10700	5600
1145	42nd BImSchV scope	7300	15600
1146	42nd BImSchV scope	59400	30600
1147	42nd BImSchV scope	14400	970
1148	42nd BImSchV scope	610	370
1149	42nd BImSchV scope	<DL	200
1150	42nd BImSchV scope	810	250
1151	42nd BImSchV scope	30	50
1152	42nd BImSchV scope	880	6600
1153	42nd BImSchV scope	490	480
1154	42nd BImSchV scope	180	50
1155	42nd BImSchV scope	4600	3900
1156	42nd BImSchV scope	110	170
1157	other industrial site	17400	13800
1158	other industrial site	30000	75600
1159	other industrial site	22800	10800
1160	other industrial site	16800	18600
1161	other industrial site	<DL	<DL
1162	other industrial site	7900	7100
1163	other industrial site	<DL	<DL
1164	other industrial site	<DL	<DL
1165	other industrial site	<DL	<DL
1166	other industrial site	<DL	<DL

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1167	other industrial site	<DL	<DL
1168	other industrial site	<DL	<DL
1169	42nd BImSchV scope	1010	90
1170	42nd BImSchV scope	7000	3600
1171	42nd BImSchV scope	16800	6000
1172	42nd BImSchV scope	24600	6800
1173	42nd BImSchV scope	4400	3700
1174	42nd BImSchV scope	640	1600
1175	42nd BImSchV scope	3100	2300
1176	other industrial site	19800	3200
1177	42nd BImSchV scope	16200	27000
1178	42nd BImSchV scope	30	<DL
1179	42nd BImSchV scope	5100	10800
1180	42nd BImSchV scope	70200	27000
1181	42nd BImSchV scope	5400	1660
1182	42nd BImSchV scope	21600	59400
1183	42nd BImSchV scope	5400	3000
1184	42nd BImSchV scope	820	540
1185	42nd BImSchV scope	570	300
1186	42nd BImSchV scope	3000	4800
1187	42nd BImSchV scope	80	100
1188	42nd BImSchV scope	1690	580
1189	42nd BImSchV scope	<DL	<DL
1190	42nd BImSchV scope	440	190
1191	42nd BImSchV scope	340	140000
1192	42nd BImSchV scope	100	160
1193	42nd BImSchV scope	<DL	<DL
1194	42nd BImSchV scope	<DL	<DL
1195	42nd BImSchV scope	<DL	<DL
1196	42nd BImSchV scope	380	490
1197	42nd BImSchV scope	1020	1360
1198	42nd BImSchV scope	260	390
1199	42nd BImSchV scope	1430	540
1200	42nd BImSchV scope	960	na
1201	42nd BImSchV scope	520	480
1202	42nd BImSchV scope	360	260
1203	42nd BImSchV scope	2300	na
1204	42nd BImSchV scope	980	820
1205	42nd BImSchV scope	200	120
1206	42nd BImSchV scope	<DL	<DL
1207	42nd BImSchV scope	21600	21600
1208	42nd BImSchV scope	1180	<300000
1209	42nd BImSchV scope	3900	870
1210	42nd BImSchV scope	1140	180
1211	42nd BImSchV scope	16200	16200

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1212	42nd BImSchV scope	<DL	10
1213	42nd BImSchV scope	2400	640
1214	other industrial site	5400	16200
1215	other industrial site	140	320
1216	other industrial site	110	110
1217	other industrial site	<DL	<DL
1218	42nd BImSchV scope	300	520
1219	42nd BImSchV scope	390	270
1220	42nd BImSchV scope	220	370
1221	42nd BImSchV scope	560	480
1222	42nd BImSchV scope	120	370
1223	42nd BImSchV scope	840	870
1224	42nd BImSchV scope	320	400
1225	42nd BImSchV scope	1160	980
1226	42nd BImSchV scope	180	220
1227	42nd BImSchV scope	1580	1690
1228	other industrial site	510	1260
1229	42nd BImSchV scope	180	120
1230	42nd BImSchV scope	TNTC	205200
1231	42nd BImSchV scope	1160	7000
1232	42nd BImSchV scope	32400	26400
1233	42nd BImSchV scope	21600	5400
1234	42nd BImSchV scope	21600	5100
1235	42nd BImSchV scope	5200	6700
1236	42nd BImSchV scope	37800	75000
1237	42nd BImSchV scope	580	2300
1238	42nd BImSchV scope	670	10200
1239	42nd BImSchV scope	50	290
1240	42nd BImSchV scope	640	21000
1241	42nd BImSchV scope	10	<DL
1242	42nd BImSchV scope	120	670
1243	42nd BImSchV scope	10	10
1244	other industrial site	102600	135000
1245	42nd BImSchV scope	100	50
1246	42nd BImSchV scope	830	2600
1247	42nd BImSchV scope	2800	1320
1248	42nd BImSchV scope	4500	6400
1249	other industrial site	3600	3100
1250	42nd BImSchV scope	14400	16200
1251	42nd BImSchV scope	320	140
1252	42nd BImSchV scope	400	160
1253	42nd BImSchV scope	350	194400
1254	42nd BImSchV scope	80	420
1255	other industrial site	<DL	10800
1256	other industrial site	TNTC	TNTC

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1257	other industrial site	5800	3600
1258	42nd BImSchV scope	4200	27000
1259	42nd BImSchV scope	18000	226800
1260	42nd BImSchV scope	<DL	40
1261	42nd BImSchV scope	710	21600
1262	42nd BImSchV scope	3100	TNTC
1263	42nd BImSchV scope	340	480
1264	42nd BImSchV scope	5100	32400
1265	42nd BImSchV scope	75600	583200
1266	42nd BImSchV scope	1070	410
1267	42nd BImSchV scope	205200	TNTC
1268	42nd BImSchV scope	216000	TNTC
1269	other industrial site	4400	5900
1270	42nd BImSchV scope	<DL	<DL
1271	42nd BImSchV scope	160	20
1272	42nd BImSchV scope	300	220
1273	42nd BImSchV scope	40	120
1274	42nd BImSchV scope	320	840
1275	42nd BImSchV scope	320	120
1276	42nd BImSchV scope	<DL	<DL
1277	42nd BImSchV scope	40	90
1278	42nd BImSchV scope	20	20
1279	42nd BImSchV scope	180	200
1280	42nd BImSchV scope	230	140
1281	42nd BImSchV scope	<DL	<DL
1282	42nd BImSchV scope	80	30
1283	42nd BImSchV scope	250	250
1284	42nd BImSchV scope	870	430
1285	42nd BImSchV scope	210	280
1286	42nd BImSchV scope	340	240
1287	42nd BImSchV scope	190	730
1288	42nd BImSchV scope	40	150
1289	42nd BImSchV scope	30	160
1290	42nd BImSchV scope	20	<DL
1291	42nd BImSchV scope	360	130
1292	42nd BImSchV scope	460	2000
1293	42nd BImSchV scope	330	50
1294	42nd BImSchV scope	190	10
1295	42nd BImSchV scope	210	180
1296	42nd BImSchV scope	<DL	<DL
1297	42nd BImSchV scope	<DL	50
1298	42nd BImSchV scope	<DL	<DL
1299	42nd BImSchV scope	50	70
1300	42nd BImSchV scope	<DL	20
1301	42nd BImSchV scope	8100	60

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1302	42nd BImSchV scope	230	80
1303	42nd BImSchV scope	320	100
1304	42nd BImSchV scope	10	<DL
1305	42nd BImSchV scope	2800	310
1306	42nd BImSchV scope	480	630
1307	42nd BImSchV scope	710	4200
1308	42nd BImSchV scope	10	<DL
1309	42nd BImSchV scope	270000	178200
1310	42nd BImSchV scope	10800	4700
1311	42nd BImSchV scope	150	180
1312	42nd BImSchV scope	<DL	<DL
1313	42nd BImSchV scope	<DL	<DL
1314	other industrial site	10400	3700
1315	other industrial site	1980	1040
1316	other industrial site	4100	4200
1317	other industrial site	9000	5300
1318	other industrial site	2500	1900
1319	other industrial site	8600	3400
1320	42nd BImSchV scope	950	460
1321	42nd BImSchV scope	2100	9200
1322	42nd BImSchV scope	10500	4500
1323	42nd BImSchV scope	640	9600
1324	other industrial site	1490	3100
1325	42nd BImSchV scope	2300	12000
1326	42nd BImSchV scope	<DL	<DL
1327	42nd BImSchV scope	1070	710
1328	42nd BImSchV scope	9500	4500
1329	42nd BImSchV scope	2300	800
1330	42nd BImSchV scope	20	<DL
1331	42nd BImSchV scope	<DL	<DL
1332	42nd BImSchV scope	<DL	<DL
1333	42nd BImSchV scope	10	<DL
1334	42nd BImSchV scope	270	650
1335	42nd BImSchV scope	18000	14400
1336	other industrial site	5400	13200
1337	other industrial site	4400	3600
1338	other industrial site	1310	151200
1339	other industrial site	2400	6800
1340	42nd BImSchV scope	1500	1700
1341	42nd BImSchV scope	18000	17400
1342	42nd BImSchV scope	710	1900
1343	42nd BImSchV scope	205000	194000
1344	42nd BImSchV scope	162000	81000
1345	42nd BImSchV scope	5100	13200
1346	42nd BImSchV scope	189000	13800

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1347	42nd BImSchV scope	162000	81000
1348	42nd BImSchV scope	250	100
1349	42nd BImSchV scope	6300	13800
1350	42nd BImSchV scope	91800	129600
1351	42nd BImSchV scope	1500	3100
1352	42nd BImSchV scope	1100	29400
1353	42nd BImSchV scope	20	30
1354	42nd BImSchV scope	3000	2000
1355	42nd BImSchV scope	710	11400
1356	42nd BImSchV scope	<DL	<DL
1357	42nd BImSchV scope	3200	5200
1358	42nd BImSchV scope	3600	2200
1359	42nd BImSchV scope	486000	135000
1360	other industrial site	102600	194400
1361	42nd BImSchV scope	1500	530
1362	42nd BImSchV scope	12400	4400
1363	42nd BImSchV scope	10600	4700
1364	42nd BImSchV scope	3400	4700
1365	42nd BImSchV scope	5100	2400
1366	42nd BImSchV scope	10	<DL
1367	42nd BImSchV scope	720	7200
1368	42nd BImSchV scope	660	1700
1369	42nd BImSchV scope	10	20
1370	other industrial site	520	5100
1371	42nd BImSchV scope	<DL	<DL
1372	42nd BImSchV scope	2300	1700
1373	42nd BImSchV scope	10800	27000
1374	42nd BImSchV scope	21600	32400
1375	42nd BImSchV scope	720	480
1376	42nd BImSchV scope	2400	1670
1377	42nd BImSchV scope	2400	1060
1378	42nd BImSchV scope	430	980
1379	42nd BImSchV scope	1820	2360
1380	42nd BImSchV scope	360	190
1381	42nd BImSchV scope	280800	561600
1382	42nd BImSchV scope	<DL	10
1383	42nd BImSchV scope	<DL	20
1384	42nd BImSchV scope	2100	TNTC
1385	42nd BImSchV scope	210	120
1386	42nd BImSchV scope	10800	48600
1387	42nd BImSchV scope	40	60
1388	42nd BImSchV scope	20	100
1389	42nd BImSchV scope	920	1460
1390	42nd BImSchV scope	440	270
1391	42nd BImSchV scope	32400	32400

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1392	42nd BImSchV scope	290	5400
1393	42nd BImSchV scope	237600	340200
1394	42nd BImSchV scope	253800	302400
1395	42nd BImSchV scope	324000	253800
1396	other industrial site	600	890
1397	42nd BImSchV scope	550	1530
1398	42nd BImSchV scope	120	110
1399	42nd BImSchV scope	5200	8000
1400	42nd BImSchV scope	<DL	<DL
1401	42nd BImSchV scope	31200	64800
1402	42nd BImSchV scope	6800	740
1403	42nd BImSchV scope	113400	10800
1404	42nd BImSchV scope	1150	1680
1405	42nd BImSchV scope	390	20
1406	other industrial site	1800	1820
1407	42nd BImSchV scope	16200	16200
1408	42nd BImSchV scope	156600	37800
1409	42nd BImSchV scope	27000	21600
1410	42nd BImSchV scope	48600	113400
1411	42nd BImSchV scope	70	10
1412	42nd BImSchV scope	280	<DL
1413	42nd BImSchV scope	460	1810
1414	42nd BImSchV scope	42	26
1415	42nd BImSchV scope	760	5400
1416	42nd BImSchV scope	28	22
1417	42nd BImSchV scope	180	390
1418	42nd BImSchV scope	170	70
1419	42nd BImSchV scope	<DL	10
1420	42nd BImSchV scope	980	16200
1421	42nd BImSchV scope	520	1800
1422	42nd BImSchV scope	350	40
1423	42nd BImSchV scope	460	1100
1424	42nd BImSchV scope	290	430
1425	42nd BImSchV scope	470	32
1426	42nd BImSchV scope	1030	16200
1427	42nd BImSchV scope	390	240
1428	42nd BImSchV scope	90	10800
1429	42nd BImSchV scope	40	<DL
1430	42nd BImSchV scope	640	340
1431	42nd BImSchV scope	<DL	<DL
1432	42nd BImSchV scope	410	280
1433	42nd BImSchV scope	<DL	<DL
1434	42nd BImSchV scope	10	<DL
1435	42nd BImSchV scope	50	30
1436	42nd BImSchV scope	30	90

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1437	42nd BImSchV scope	<DL	<DL
1438	42nd BImSchV scope	<DL	60
1439	42nd BImSchV scope	<DL	<DL
1440	42nd BImSchV scope	160	70
1441	42nd BImSchV scope	<DL	<DL
1442	42nd BImSchV scope	200	310
1443	42nd BImSchV scope	<DL	<DL
1444	42nd BImSchV scope	<DL	<DL
1445	other industrial site	660	520
1446	other industrial site	11400	21600
1447	other industrial site	2100	520
1448	other industrial site	700	640
1449	other industrial site	210	<DL
1450	other industrial site	950	1120
1451	42nd BImSchV scope	320	2700
1452	42nd BImSchV scope	300	260
1453	42nd BImSchV scope	60	80
1454	42nd BImSchV scope	130	140
1455	42nd BImSchV scope	390	340
1456	42nd BImSchV scope	70	40
1457	42nd BImSchV scope	280	180
1458	42nd BImSchV scope	170	110
1459	42nd BImSchV scope	210	40
1460	42nd BImSchV scope	130	40
1461	42nd BImSchV scope	960	1370
1462	42nd BImSchV scope	3900	10800
1463	42nd BImSchV scope	15600	1070
1464	other industrial site	1270	1040
1465	other industrial site	180	940
1466	42nd BImSchV scope	4300	4500
1467	42nd BImSchV scope	<DL	<DL
1468	42nd BImSchV scope	<DL	<DL
1469	42nd BImSchV scope	20	<DL
1470	42nd BImSchV scope	16800	3400
1471	other industrial site	237600	453600
1472	42nd BImSchV scope	560	2100
1473	42nd BImSchV scope	<DL	20
1474	42nd BImSchV scope	130	40
1475	42nd BImSchV scope	180	30
1476	42nd BImSchV scope	2300	2640
1477	42nd BImSchV scope	54000	6400
1478	42nd BImSchV scope	59400	184800
1479	42nd BImSchV scope	16200	10500
1480	42nd BImSchV scope	15600	80
1481	42nd BImSchV scope	8400	5400

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1482	42nd BImSchV scope	10200	1600
1483	42nd BImSchV scope	27000	27000
1484	42nd BImSchV scope	97200	388800
1485	42nd BImSchV scope	<DL	<DL
1486	42nd BImSchV scope	81000	86400
1487	42nd BImSchV scope	3100	5400
1488	42nd BImSchV scope	660	580
1489	42nd BImSchV scope	102600	102600
1490	42nd BImSchV scope	3900	2900
1491	42nd BImSchV scope	13200	16200
1492	other industrial site	16200	21600
1493	42nd BImSchV scope	680	2500
1494	42nd BImSchV scope	70200	70200
1495	42nd BImSchV scope	5400	8500
1496	42nd BImSchV scope	302400	920
1497	42nd BImSchV scope	259200	22800
1498	42nd BImSchV scope	5400	10800
1499	42nd BImSchV scope	2700	48600
1500	42nd BImSchV scope	640	22800
1501	42nd BImSchV scope	4200	TNTC
1502	42nd BImSchV scope	680	260
1503	42nd BImSchV scope	27000	64800
1504	42nd BImSchV scope	320	190
1505	42nd BImSchV scope	1800	600
1506	42nd BImSchV scope	900	1500
1507	42nd BImSchV scope	90	510
1508	42nd BImSchV scope	27000	21000
1509	42nd BImSchV scope	16200	31800
1510	other industrial site	13400	97200
1511	42nd BImSchV scope	<DL	<DL
1512	42nd BImSchV scope	20	30
1513	42nd BImSchV scope	<DL	20
1514	42nd BImSchV scope	<DL	<DL
1515	42nd BImSchV scope	21600	21600
1516	42nd BImSchV scope	20	320
1517	42nd BImSchV scope	<DL	<DL
1518	42nd BImSchV scope	<DL	<DL
1519	42nd BImSchV scope	10800	16200
1520	42nd BImSchV scope	3200	43200
1521	42nd BImSchV scope	<DL	<DL
1522	42nd BImSchV scope	32400	37800
1523	42nd BImSchV scope	27000	10800
1524	other industrial site	4900	21600
1525	other industrial site	<DL	<DL
1526	other industrial site	20	110

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1527	other industrial site	40	440
1528	other industrial site	<DL	20
1529	42nd BImSchV scope	160	3400
1530	other industrial site	280	1100
1531	42nd BImSchV scope	290	40
1532	42nd BImSchV scope	720	870
1533	42nd BImSchV scope	166400	TNTC
1534	42nd BImSchV scope	50	20
1535	42nd BImSchV scope	260	30
1536	42nd BImSchV scope	<DL	<DL
1537	42nd BImSchV scope	<DL	90
1538	42nd BImSchV scope	2800	2600
1539	42nd BImSchV scope	4500	4700
1540	42nd BImSchV scope	3000	4800
1541	42nd BImSchV scope	4300	3200
1542	42nd BImSchV scope	420	6600
1543	other industrial site	450	1800
1544	42nd BImSchV scope	170	940
1545	42nd BImSchV scope	14400	81000
1546	42nd BImSchV scope	140	<DL
1547	42nd BImSchV scope	260	1090
1548	42nd BImSchV scope	600	2700
1549	42nd BImSchV scope	240	4800
1550	42nd BImSchV scope	250	2100
1551	42nd BImSchV scope	260	2700
1552	42nd BImSchV scope	350	740
1553	42nd BImSchV scope	80	190
1554	42nd BImSchV scope	130	490
1555	42nd BImSchV scope	40	11400
1556	42nd BImSchV scope	850	1600
1557	42nd BImSchV scope	280	470
1558	42nd BImSchV scope	240	690
1559	42nd BImSchV scope	280	1400
1560	42nd BImSchV scope	290	640
1561	42nd BImSchV scope	2600	59400
1562	42nd BImSchV scope	370	TNTC
1563	42nd BImSchV scope	330	3800
1564	42nd BImSchV scope	440	40
1565	42nd BImSchV scope	870	2200
1566	42nd BImSchV scope	20	80
1567	42nd BImSchV scope	9600	2700
1568	42nd BImSchV scope	<DL	40
1569	42nd BImSchV scope	31800	1750
1570	42nd BImSchV scope	<DL	110
1571	42nd BImSchV scope	<DL	20

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1572	42nd BImSchV scope	<DL	30
1573	42nd BImSchV scope	<DL	<DL
1574	42nd BImSchV scope	<DL	<DL
1575	42nd BImSchV scope	790	2300
1576	42nd BImSchV scope	10800	17400
1577	other industrial site	250	190
1578	other industrial site	22800	15000
1579	other industrial site	1250	1100
1580	other industrial site	4100	4100
1581	other industrial site	80	30
1582	other industrial site	5100	3700
1583	42nd BImSchV scope	420	500
1584	42nd BImSchV scope	113400	199800
1585	42nd BImSchV scope	183600	TNTC
1586	42nd BImSchV scope	297000	TNTC
1587	42nd BImSchV scope	486000	TNTC
1588	42nd BImSchV scope	940	8900
1589	other industrial site	2800	3600
1590	42nd BImSchV scope	390	640
1591	42nd BImSchV scope	<DL	10
1592	42nd BImSchV scope	27000	64800
1593	42nd BImSchV scope	480	120
1594	42nd BImSchV scope	720	520
1595	42nd BImSchV scope	410	TNTC
1596	42nd BImSchV scope	30	<DL
1597	42nd BImSchV scope	3000	9000
1598	42nd BImSchV scope	30	420
1599	42nd BImSchV scope	200	230
1600	42nd BImSchV scope	48600	91800
1601	42nd BImSchV scope	37800	32400
1602	42nd BImSchV scope	129600	70200
1603	42nd BImSchV scope	360	510
1604	42nd BImSchV scope	760	300
1605	42nd BImSchV scope	670	910
1606	42nd BImSchV scope	16200	3400
1607	42nd BImSchV scope	360	260
1608	42nd BImSchV scope	1800	2300
1609	42nd BImSchV scope	1000	50
1610	42nd BImSchV scope	32400	16200
1611	42nd BImSchV scope	37800	43200
1612	42nd BImSchV scope	<DL	30
1613	42nd BImSchV scope	<DL	<DL
1614	42nd BImSchV scope	50	230
1615	42nd BImSchV scope	5400	16200
1616	42nd BImSchV scope	3200	5400

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1617	42nd BImSchV scope	340	40
1618	42nd BImSchV scope	2700	3600
1619	42nd BImSchV scope	1330	3800
1620	42nd BImSchV scope	<DL	<DL
1621	42nd BImSchV scope	590	50
1622	42nd BImSchV scope	420	1500
1623	42nd BImSchV scope	<DL	<DL
1624	42nd BImSchV scope	630	170
1625	42nd BImSchV scope	16200	5600
1626	42nd BImSchV scope	3700	5400
1627	42nd BImSchV scope	870	16200
1628	42nd BImSchV scope	<DL	<DL
1629	other industrial site	10800	27000
1630	other industrial site	16200	16200
1631	other industrial site	790	2100
1632	other industrial site	118800	TNTC
1633	other industrial site	10800	97200
1634	other industrial site	<DL	20
1635	other industrial site	21600	75600
1636	other industrial site	<DL	600
1637	other industrial site	6000	81000
1638	42nd BImSchV scope	15600	10200
1639	42nd BImSchV scope	<DL	<DL
1640	42nd BImSchV scope	970	2600
1641	42nd BImSchV scope	<DL	10
1642	42nd BImSchV scope	<DL	<DL
1643	other industrial site	70200	70200
1644	42nd BImSchV scope	190	890
1645	42nd BImSchV scope	4800	3700
1646	42nd BImSchV scope	3900	4600
1647	42nd BImSchV scope	5600	4500
1648	42nd BImSchV scope	7800	17400
1649	42nd BImSchV scope	91800	135000
1650	42nd BImSchV scope	118800	124200
1651	42nd BImSchV scope	290	1340
1652	42nd BImSchV scope	900	160
1653	42nd BImSchV scope	64800	2200
1654	42nd BImSchV scope	55400	70200
1655	42nd BImSchV scope	320	100
1656	42nd BImSchV scope	86900	28200
1657	42nd BImSchV scope	6400	1260
1658	other industrial site	183600	102600
1659	42nd BImSchV scope	<DL	<DL
1660	42nd BImSchV scope	100	90
1661	42nd BImSchV scope	3200	4900

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1662	42nd BImSchV scope	1700	4000
1663	42nd BImSchV scope	570	9000
1664	42nd BImSchV scope	290	4300
1665	42nd BImSchV scope	90	20
1666	42nd BImSchV scope	780	1040
1667	42nd BImSchV scope	30	390
1668	42nd BImSchV scope	1300	7900
1669	other industrial site	70	10
1670	42nd BImSchV scope	12000	6700
1671	other industrial site	7500	9600
1672	42nd BImSchV scope	1800	640
1673	42nd BImSchV scope	50	50
1674	42nd BImSchV scope	2160	108000
1675	42nd BImSchV scope	43200	43200
1676	42nd BImSchV scope	30240	73200
1677	42nd BImSchV scope	2500	830
1678	42nd BImSchV scope	10800	<DL
1679	42nd BImSchV scope	100	1300
1680	42nd BImSchV scope	5800	2100
1681	42nd BImSchV scope	59400	48600
1682	42nd BImSchV scope	86400	27000
1683	42nd BImSchV scope	<DL	10
1684	42nd BImSchV scope	880	1360
1685	42nd BImSchV scope	<DL<	<DL
1686	42nd BImSchV scope	<DL	<DL<
1687	42nd BImSchV scope	40	20
1688	42nd BImSchV scope	8800	15600
1689	42nd BImSchV scope	194400	97200
1690	42nd BImSchV scope	2700	20
1691	42nd BImSchV scope	167400	113400
1692	42nd BImSchV scope	118800	113400
1693	other industrial site	28200	6900
1694	42nd BImSchV scope	19200	TNTC
1695	42nd BImSchV scope	610	1050
1696	42nd BImSchV scope	140	80
1697	42nd BImSchV scope	170	70
1698	42nd BImSchV scope	630	370
1699	42nd BImSchV scope	910	1900
1700	42nd BImSchV scope	580	720
1701	42nd BImSchV scope	190	290
1702	42nd BImSchV scope	180	210
1703	42nd BImSchV scope	<DL	10
1704	42nd BImSchV scope	<DL	<DL
1705	42nd BImSchV scope	60	40
1706	42nd BImSchV scope	240	150

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1707	42nd BImSchV scope	230	620
1708	42nd BImSchV scope	10200	12600
1709	42nd BImSchV scope	850	710
1710	42nd BImSchV scope	250	120
1711	42nd BImSchV scope	2600	13200
1712	42nd BImSchV scope	290	3500
1713	42nd BImSchV scope	300	1150
1714	42nd BImSchV scope	480	100
1715	42nd BImSchV scope	230	390
1716	42nd BImSchV scope	460	210
1717	42nd BImSchV scope	3900	4200
1718	42nd BImSchV scope	4700	3600
1719	42nd BImSchV scope	4500	3000
1720	42nd BImSchV scope	270	30
1721	42nd BImSchV scope	20	10
1722	42nd BImSchV scope	<DL	10
1723	42nd BImSchV scope	<DL	<DL
1724	42nd BImSchV scope	60	100
1725	42nd BImSchV scope	<DL	<DL
1726	42nd BImSchV scope	<DL	<DL
1727	42nd BImSchV scope	2900	2200
1728	42nd BImSchV scope	4900	5400
1729	other industrial site	790	620
1730	other industrial site	12600	16800
1731	other industrial site	7200	7200
1732	other industrial site	830	790
1733	other industrial site	2800	1330
1734	other industrial site	5400	3700
1735	other industrial site	<DL	<DL
1736	other industrial site	<DL	<DL
1737	other industrial site	<DL	10
1738	other industrial site	<DL	<DL
1739	other industrial site	<DL	<DL
1740	other industrial site	<DL	<DL
1741	42nd BImSchV scope	10	10
1742	42nd BImSchV scope	620	660
1743	42nd BImSchV scope	12000	43200
1744	42nd BImSchV scope	189000	TNTC
1745	42nd BImSchV scope	27000	75600
1746	42nd BImSchV scope	32400	59400
1747	42nd BImSchV scope	2500	11400
1748	42nd BImSchV scope	<DL	2500
1749	42nd BImSchV scope	120	1090
1750	42nd BImSchV scope	<DL	<DL
1751	42nd BImSchV scope	<DL	<DL

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1752	42nd BImSchV scope	<DL	20
1753	42nd BImSchV scope	1860	7200
1754	42nd BImSchV scope	324000	8640
1755	42nd BImSchV scope	102600	91800
1756	other industrial site	9000	2800
1757	42nd BImSchV scope	110	1850
1758	42nd BImSchV scope	21600	10200
1759	42nd BImSchV scope	440	40
1760	42nd BImSchV scope	140	470
1761	42nd BImSchV scope	<DL	10
1762	42nd BImSchV scope	<DL	170
1763	42nd BImSchV scope	830	179
1764	42nd BImSchV scope	15600	11400
1765	42nd BImSchV scope	27600	26400
1766	42nd BImSchV scope	21000	24000
1767	42nd BImSchV scope	36000	23400
1768	42nd BImSchV scope	80	1780
1769	42nd BImSchV scope	940	810
1770	42nd BImSchV scope	<DL	10
1771	42nd BImSchV scope	3100	1690
1772	42nd BImSchV scope	24600	6600
1773	42nd BImSchV scope	2500	2900
1774	42nd BImSchV scope	950	900
1775	42nd BImSchV scope	170	40
1776	42nd BImSchV scope	660	10
1777	42nd BImSchV scope	1900	10800
1778	other industrial site	151200	140400
1779	other industrial site	<DL	<DL
1780	other industrial site	TNTC	TNTC
1781	other industrial site	145800	118800
1782	other industrial site	260	1780
1783	other industrial site	44400	54500
1784	other industrial site	33000	64800
1785	other industrial site	40	910
1786	other industrial site	4500	5300
1787	other industrial site	2600	1500
1788	42nd BImSchV scope	26400	6100
1789	42nd BImSchV scope	410	2500
1790	42nd BImSchV scope	4300	10100
1791	42nd BImSchV scope	7200	12700
1792	42nd BImSchV scope	<DL	10
1793	42nd BImSchV scope	3300	5400
1794	42nd BImSchV scope	240	1620
1795	42nd BImSchV scope	890	3800
1796	42nd BImSchV scope	4300	5800

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1797	42nd BImSchV scope	1500	5400
1798	42nd BImSchV scope	560	3800
1799	42nd BImSchV scope	50	350
1800	42nd BImSchV scope	2400	17400
1801	42nd BImSchV scope	240	2100
1802	42nd BImSchV scope	20	40
1803	42nd BImSchV scope	1190	3700
1804	42nd BImSchV scope	40	20
1805	42nd BImSchV scope	<DL	<DL
1806	42nd BImSchV scope	194400	270000
1807	42nd BImSchV scope	10	250
1808	42nd BImSchV scope	20	<DL
1809	42nd BImSchV scope	102600	118800
1810	42nd BImSchV scope	590	108000
1811	42nd BImSchV scope	50	10
1812	42nd BImSchV scope	300	<DL
1813	42nd BImSchV scope	81000	190
1814	other industrial site	75600	118800
1815	other industrial site	2900	4400
1816	other industrial site	560	690
1817	other industrial site	2300	3000
1818	42nd BImSchV scope	290	3600
1819	42nd BImSchV scope	1800	1110
1820	42nd BImSchV scope	900	1900
1821	42nd BImSchV scope	80	40
1822	42nd BImSchV scope	780	500
1823	42nd BImSchV scope	110	270
1824	42nd BImSchV scope	80	800
1825	42nd BImSchV scope	110	70
1826	42nd BImSchV scope	100	290
1827	42nd BImSchV scope	390	2500
1828	42nd BImSchV scope	<DL	20
1829	42nd BImSchV scope	<DL	30
1830	42nd BImSchV scope	50	50
1831	42nd BImSchV scope	640	1430
1832	42nd BImSchV scope	100	160
1833	42nd BImSchV scope	1800	710
1834	42nd BImSchV scope	10	20
1835	42nd BImSchV scope	10	<DL
1836	42nd BImSchV scope	1840	22800
1837	42nd BImSchV scope	40	720
1838	42nd BImSchV scope	<DL	9
1839	42nd BImSchV scope	<DL	10
1840	42nd BImSchV scope	<DL	<DL
1841	42nd BImSchV scope	<DL	20

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1842	42nd BImSchV scope	19800	75600
1843	42nd BImSchV scope	17400	3900
1844	42nd BImSchV scope	10	<DL
1845	42nd BImSchV scope	<DL	<DL
1846	42nd BImSchV scope	<DL	<DL
1847	42nd BImSchV scope	<DL	<DL
1848	42nd BImSchV scope	<DL	<DL
1849	42nd BImSchV scope	<DL	<DL
1850	other industrial site	5000	4400
1851	other industrial site	21000	19800
1852	other industrial site	1700	1900
1853	other industrial site	41400	13200
1854	other industrial site	390	320
1855	other industrial site	420	740
1856	42nd BImSchV scope	2700	1120
1857	42nd BImSchV scope	75600	24000
1858	42nd BImSchV scope	54000	40800
1859	42nd BImSchV scope	2320	4800
1860	42nd BImSchV scope	530	450
1861	42nd BImSchV scope	70200	17400
1862	other industrial site	8200	5800
1863	42nd BImSchV scope	1900	43200
1864	42nd BImSchV scope	10800	1800
1865	42nd BImSchV scope	1200	2000
1866	42nd BImSchV scope	70200	102600
1867	42nd BImSchV scope	<DL	<DL
1868	42nd BImSchV scope	7200	27000
1869	42nd BImSchV scope	10800	1800
1870	42nd BImSchV scope	1400	980
1871	42nd BImSchV scope	10	170
1872	42nd BImSchV scope	130	290
1873	42nd BImSchV scope	60	240
1874	42nd BImSchV scope	150	150
1875	42nd BImSchV scope	170	210
1876	42nd BImSchV scope	20	80
1877	42nd BImSchV scope	140	120
1878	42nd BImSchV scope	40	130
1879	42nd BImSchV scope	100	180
1880	42nd BImSchV scope	80	360
1881	42nd BImSchV scope	340	110
1882	42nd BImSchV scope	<DL	<DL
1883	42nd BImSchV scope	170	170
1884	42nd BImSchV scope	360	8400
1885	42nd BImSchV scope	<DL	<DL
1886	42nd BImSchV scope	<DL	<DL

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1887	42nd BImSchV scope	10800	64800
1888	42nd BImSchV scope	1500	1120
1889	42nd BImSchV scope	<DL	<DL
1890	42nd BImSchV scope	<DL	200
1891	other industrial site	2200	10800
1892	other industrial site	20	90
1893	other industrial site	16200	32400
1894	42nd BImSchV scope	55800	151200
1895	42nd BImSchV scope	<DL	<DL
1896	42nd BImSchV scope	<DL	<DL
1897	42nd BImSchV scope	<DL	<DL
1898	42nd BImSchV scope	1700	2100
1899	42nd BImSchV scope	2600	1500
1900	42nd BImSchV scope	11300	27600
1901	42nd BImSchV scope	na	3300
1902	42nd BImSchV scope	na	20
1903	42nd BImSchV scope	na	410
1904	42nd BImSchV scope	na	2600
1905	42nd BImSchV scope	na	39000
1906	42nd BImSchV scope	na	2700
1907	42nd BImSchV scope	na	3500
1908	42nd BImSchV scope	na	60
1909	42nd BImSchV scope	na	970
1910	other industrial site	na	118800
1911	42nd BImSchV scope	360	760
1912	42nd BImSchV scope	10	50
1913	42nd BImSchV scope	170	30
1914	42nd BImSchV scope	50	<DL
1915	42nd BImSchV scope	135000	1800
1916	42nd BImSchV scope	5400	10800
1917	42nd BImSchV scope	4100	1500
1918	42nd BImSchV scope	30000	14400
1919	42nd BImSchV scope	<DL	<DL
1920	42nd BImSchV scope	1030	2200
1921	42nd BImSchV scope	120	680
1922	42nd BImSchV scope	750	200
1923	42nd BImSchV scope	3200	3400
1924	42nd BImSchV scope	1300	1010
1925	other industrial site	TNTC	TNTC
1926	other industrial site	44400	75600
1927	42nd BImSchV scope	22200	1900
1928	42nd BImSchV scope	14400	13200
1929	42nd BImSchV scope	10900	4800
1930	other industrial site	39600	21000
1931	42nd BImSchV scope	27000	490

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1932	42nd BImSchV scope	4300	2200
1933	42nd BImSchV scope	124200	4200
1934	42nd BImSchV scope	59400	81000
1935	42nd BImSchV scope	48600	10800
1936	42nd BImSchV scope	237600	124200
1937	42nd BImSchV scope	<DL	<DL
1938	42nd BImSchV scope	167400	140400
1939	42nd BImSchV scope	260	290
1940	42nd BImSchV scope	113400	10
1941	42nd BImSchV scope	91800	27000
1942	42nd BImSchV scope	<DL	<DL
1943	42nd BImSchV scope	110	60
1944	42nd BImSchV scope	240	130
1945	42nd BImSchV scope	10	<DL
1946	other industrial site	21600	21600
1947	42nd BImSchV scope	970	9600
1948	other industrial site	780	1200
1949	42nd BImSchV scope	TNTC	TNTC
1950	42nd BImSchV scope	370	540
1951	42nd BImSchV scope	1800	350
1952	42nd BImSchV scope	410	190
1953	42nd BImSchV scope	130	130
1954	42nd BImSchV scope	50	220
1955	42nd BImSchV scope	90	50
1956	42nd BImSchV scope	150	50
1957	42nd BImSchV scope	20	<DL
1958	42nd BImSchV scope	<DL	<DL
1959	42nd BImSchV scope	30	60
1960	42nd BImSchV scope	30	50
1961	42nd BImSchV scope	540	110
1962	42nd BImSchV scope	50	50
1963	42nd BImSchV scope	<DL	<DL
1964	42nd BImSchV scope	1020	600
1965	42nd BImSchV scope	400	230
1966	42nd BImSchV scope	170	1900
1967	42nd BImSchV scope	250	270
1968	42nd BImSchV scope	50	<DL
1969	42nd BImSchV scope	30	40
1970	42nd BImSchV scope	40	<DL
1971	42nd BImSchV scope	30	180
1972	42nd BImSchV scope	<DL	<DL
1973	42nd BImSchV scope	20	10
1974	42nd BImSchV scope	<DL	10
1975	42nd BImSchV scope	<DL	<DL
1976	42nd BImSchV scope	<DL	10

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
1977	42nd BImSchV scope	<DL	<DL
1978	42nd BImSchV scope	<DL	<DL
1979	42nd BImSchV scope	10	<DL
1980	other industrial site	135000	86400
1981	other industrial site	9900	9700
1982	other industrial site	11500	10900
1983	other industrial site	1700	1600
1984	other industrial site	1100	610
1985	other industrial site	380	500
1986	other industrial site	<DL	<DL
1987	other industrial site	<DL	<DL
1988	other industrial site	<DL	10
1989	other industrial site	10	<DL
1990	other industrial site	<DL	<DL
1991	other industrial site	<DL	<DL
1992	42nd BImSchV scope	480	410
1993	42nd BImSchV scope	5600	3900
1994	42nd BImSchV scope	8500	10000
1995	42nd BImSchV scope	TNTC	TNTC
1996	42nd BImSchV scope	75600	32400
1997	42nd BImSchV scope	TNTC	TNTC
1998	42nd BImSchV scope	TNTC	TNTC
1999	42nd BImSchV scope	560	3600
2000	42nd BImSchV scope	2250	750
2001	42nd BImSchV scope	10	<DL
2002	other industrial site	500	620
2003	42nd BImSchV scope	75600	97200
2004	42nd BImSchV scope	20	<DL
2005	42nd BImSchV scope	27000	21600
2006	42nd BImSchV scope	10800	16200
2007	42nd BImSchV scope	3100	2400
2008	42nd BImSchV scope	680	420
2009	42nd BImSchV scope	64800	129600
2010	42nd BImSchV scope	2300	680
2011	42nd BImSchV scope	10800	3300
2012	42nd BImSchV scope	21600	32400
2013	42nd BImSchV scope	2300	10800
2014	42nd BImSchV scope	10	<DL
2015	42nd BImSchV scope	<DL	<DL
2016	42nd BImSchV scope	27000	32400
2017	42nd BImSchV scope	5400	2400
2018	42nd BImSchV scope	10800	1360
2019	42nd BImSchV scope	<DL	<DL
2020	42nd BImSchV scope	16200	16200
2021	42nd BImSchV scope	2600	10800

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2022	42nd BImSchV scope	5800	10800
2023	42nd BImSchV scope	<DL	1100
2024	42nd BImSchV scope	<DL	10
2025	other industrial site	21600	75600
2026	other industrial site	<DL	1470
2027	other industrial site	<DL	<DL
2028	other industrial site	1020	4300
2029	other industrial site	16200	32400
2030	other industrial site	<DL	<DL
2031	other industrial site	<DL	40
2032	other industrial site	2400	3300
2033	42nd BImSchV scope	40	<DL
2034	42nd BImSchV scope	30	180
2035	42nd BImSchV scope	380	10800
2036	42nd BImSchV scope	2800	2100
2037	42nd BImSchV scope	64800	43200
2038	42nd BImSchV scope	16000	10800
2039	42nd BImSchV scope	390000	10800
2040	42nd BImSchV scope	860	140
2041	42nd BImSchV scope	470	220
2042	42nd BImSchV scope	40	<DL
2043	42nd BImSchV scope	<DL	<DL
2044	42nd BImSchV scope	97200	150
2045	42nd BImSchV scope	118800	27000
2046	other industrial site	370000	300000
2047	other industrial site	0	0
2048	42nd BImSchV scope	<DL	<DL
2049	42nd BImSchV scope	730	2800
2050	42nd BImSchV scope	70	760
2051	42nd BImSchV scope	520	840
2052	42nd BImSchV scope	110000	43000
2053	42nd BImSchV scope	TNTC	16200
2054	42nd BImSchV scope	480	160
2055	42nd BImSchV scope	10800	8800
2056	42nd BImSchV scope	10800	3900
2057	42nd BImSchV scope	250	310
2058	other industrial site	TNTC	TNTC
2059	other industrial site	32000	390000
2060	42nd BImSchV scope	4500	3600
2061	other industrial site	24000	16000
2062	42nd BImSchV scope	<DL	<DL
2063	42nd BImSchV scope	1530	3200
2064	42nd BImSchV scope	90	500
2065	42nd BImSchV scope	1900	2200
2066	42nd BImSchV scope	4800	2000

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2067	42nd BImSchV scope	90	410
2068	42nd BImSchV scope	2200	720
2069	42nd BImSchV scope	<DL	<DL
2070	42nd BImSchV scope	113400	189000
2071	42nd BImSchV scope	<DL	<DL
2072	42nd BImSchV scope	100	3900
2073	42nd BImSchV scope	370	440
2074	42nd BImSchV scope	210	30
2075	42nd BImSchV scope	43200	24000
2076	42nd BImSchV scope	65000	460
2077	42nd BImSchV scope	65000	135000
2078	42nd BImSchV scope	440	21600
2079	42nd BImSchV scope	TNTC	475200
2080	42nd BImSchV scope	2400	16200
2081	42nd BImSchV scope	TNTC	345600
2082	other industrial site	490	21600
2083	42nd BImSchV scope	190	40
2084	42nd BImSchV scope	50	130
2085	42nd BImSchV scope	110	110
2086	42nd BImSchV scope	60	210
2087	42nd BImSchV scope	80	120
2088	42nd BImSchV scope	140	40
2089	42nd BImSchV scope	<DL	<DL
2090	42nd BImSchV scope	<DL	<DL
2091	42nd BImSchV scope	<DL	30
2092	42nd BImSchV scope	40	10
2093	42nd BImSchV scope	330	80
2094	42nd BImSchV scope	960	130
2095	42nd BImSchV scope	<DL	<DL
2096	42nd BImSchV scope	60	100
2097	42nd BImSchV scope	110	110
2098	42nd BImSchV scope	3700	14000
2099	42nd BImSchV scope	200	190
2100	42nd BImSchV scope	10	50
2101	42nd BImSchV scope	440	10
2102	42nd BImSchV scope	100	4200
2103	42nd BImSchV scope	5700	9600
2104	42nd BImSchV scope	740	15000
2105	42nd BImSchV scope	<DL	30
2106	42nd BImSchV scope	<DL	1600
2107	42nd BImSchV scope	130	30
2108	42nd BImSchV scope	<DL	230
2109	42nd BImSchV scope	80	<DL
2110	42nd BImSchV scope	<DL	<DL
2111	42nd BImSchV scope	<DL	<DL

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2112	42nd BImSchV scope	<DL	<DL
2113	42nd BImSchV scope	<DL	<DL
2114	42nd BImSchV scope	80	<DL
2115	other industrial site	9900	10200
2116	other industrial site	1200	1150
2117	other industrial site	10900	10000
2118	other industrial site	7200	8400
2119	other industrial site	2800	1530
2120	other industrial site	1110	1070
2121	42nd BImSchV scope	560	90
2122	42nd BImSchV scope	6000	5400
2123	42nd BImSchV scope	11000	2900
2124	42nd BImSchV scope	<DL	30
2125	42nd BImSchV scope	770	2900
2126	42nd BImSchV scope	1020	2100
2127	42nd BImSchV scope	<DL	<DL
2128	42nd BImSchV scope	<DL	<DL
2129	42nd BImSchV scope	<DL	<DL
2130	42nd BImSchV scope	600	1040
2131	42nd BImSchV scope	24000	234000
2132	other industrial site	18000	2500
2133	42nd BImSchV scope	190	1900
2134	other industrial site	500	560
2135	42nd BImSchV scope	21600	27000
2136	42nd BImSchV scope	<DL	<DL
2137	42nd BImSchV scope	350	230
2138	42nd BImSchV scope	390	680
2139	42nd BImSchV scope	28000	43000
2140	42nd BImSchV scope	560	11000
2141	42nd BImSchV scope	10	<DL
2142	42nd BImSchV scope	<DL	<DL
2143	42nd BImSchV scope	13000	22000
2144	42nd BImSchV scope	86400	86400
2145	42nd BImSchV scope	97200	64800
2146	42nd BImSchV scope	9600	5400
2147	42nd BImSchV scope	<DL	220
2148	other industrial site	13000	27000
2149	other industrial site	<DL	<DL
2150	other industrial site	<DL	<DL
2151	other industrial site	5800	16000
2152	other industrial site	<DL	<DL
2153	other industrial site	179820	179820
2154	other industrial site	28800	16200
2155	other industrial site	160	260
2156	other industrial site	850	1700

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2157	42nd BImSchV scope	540000	110000
2158	42nd BImSchV scope	380000	210000
2159	42nd BImSchV scope	<DL	<DL
2160	42nd BImSchV scope	20	<DL
2161	42nd BImSchV scope	30	280
2162	42nd BImSchV scope	37200	<DL
2163	42nd BImSchV scope	810	60
2164	42nd BImSchV scope	230	50
2165	42nd BImSchV scope	<DL	<DL
2166	42nd BImSchV scope	<DL	<DL
2167	42nd BImSchV scope	30	60
2168	42nd BImSchV scope	42600	180
2169	42nd BImSchV scope	40	90
2170	42nd BImSchV scope	80	30
2171	42nd BImSchV scope	20	<DL
2172	other industrial site	140400	27000
2173	42nd BImSchV scope	760	2300
2174	42nd BImSchV scope	840	870
2175	42nd BImSchV scope	270	590
2176	42nd BImSchV scope	20	3100
2177	42nd BImSchV scope	300	300
2178	42nd BImSchV scope	21600	8900
2179	42nd BImSchV scope	16200	3300
2180	other industrial site	388800	199400
2181	other industrial site	680	490
2182	other industrial site	70200	145800
2183	42nd BImSchV scope	880	700
2184	other industrial site	730	1900
2185	42nd BImSchV scope	91800	9100
2186	42nd BImSchV scope	135000	237600
2187	42nd BImSchV scope	1600	1500
2188	42nd BImSchV scope	<DL	<DL
2189	42nd BImSchV scope	140000	206000
2190	42nd BImSchV scope	1100	510
2191	42nd BImSchV scope	400	50
2192	42nd BImSchV scope	610	81000
2193	42nd BImSchV scope	12000	11400
2194	42nd BImSchV scope	<DL	<DL
2195	42nd BImSchV scope	720	590
2196	42nd BImSchV scope	<DL	<DL
2197	42nd BImSchV scope	40	<DL
2198	other industrial site	16000	23000
2199	42nd BImSchV scope	22800	22200
2200	42nd BImSchV scope	7800	510
2201	42nd BImSchV scope	24600	8700

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2202	42nd BImSchV scope	21000	9300
2203	42nd BImSchV scope	<DL	<DL
2204	42nd BImSchV scope	1300	2400
2205	42nd BImSchV scope	100	280
2206	42nd BImSchV scope	2100	2200
2207	42nd BImSchV scope	230	290
2208	42nd BImSchV scope	9100	2900
2209	42nd BImSchV scope	590	330
2210	42nd BImSchV scope	60	40
2211	42nd BImSchV scope	10300	6500
2212	42nd BImSchV scope	2600	6900
2213	42nd BImSchV scope	650	290
2214	42nd BImSchV scope	510	290
2215	other industrial site	12600	9900
2216	42nd BImSchV scope	3200	4900
2217	42nd BImSchV scope	10	<DL
2218	42nd BImSchV scope	580	1800
2219	42nd BImSchV scope	6800	2300
2220	42nd BImSchV scope	10	10
2221	42nd BImSchV scope	480	5800
2222	42nd BImSchV scope	200	3900
2223	42nd BImSchV scope	220	2200
2224	42nd BImSchV scope	<DL	50
2225	42nd BImSchV scope	<DL	540
2226	42nd BImSchV scope	23000	21000
2227	42nd BImSchV scope	800	250
2228	42nd BImSchV scope	280	630
2229	42nd BImSchV scope	3100	2700
2230	42nd BImSchV scope	370	470
2231	42nd BImSchV scope	40	250
2232	42nd BImSchV scope	1500	5000
2233	42nd BImSchV scope	60	770
2234	42nd BImSchV scope	60	1600
2235	42nd BImSchV scope	150	70
2236	42nd BImSchV scope	590	1800
2237	42nd BImSchV scope	440	520
2238	42nd BImSchV scope	<DL	<DL
2239	42nd BImSchV scope	<DL	<DL
2240	42nd BImSchV scope	<DL	<DL
2241	42nd BImSchV scope	<DL	<DL
2242	42nd BImSchV scope	<DL	<DL
2243	42nd BImSchV scope	20	10
2244	42nd BImSchV scope	<DL	<DL
2245	42nd BImSchV scope	16000	5700
2246	42nd BImSchV scope	230	300

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2247	other industrial site	<DL	<DL
2248	other industrial site	<DL	<DL
2249	other industrial site	<DL	<DL
2250	other industrial site	<DL	<DL
2251	other industrial site	<DL	<DL
2252	other industrial site	<DL	<DL
2253	42nd BImSchV scope	10	50
2254	42nd BImSchV scope	6000	3900
2255	42nd BImSchV scope	18000	8300
2256	42nd BImSchV scope	43000	71000
2257	42nd BImSchV scope	6700	7800
2258	42nd BImSchV scope	190000	61000
2259	42nd BImSchV scope	4300	6200
2260	42nd BImSchV scope	160	310
2261	42nd BImSchV scope	10	40
2262	42nd BImSchV scope	170	310
2263	42nd BImSchV scope	20	110
2264	42nd BImSchV scope	70	50
2265	42nd BImSchV scope	170	290
2266	42nd BImSchV scope	40	30
2267	42nd BImSchV scope	280	350
2268	42nd BImSchV scope	280	320
2269	42nd BImSchV scope	2300	4700
2270	42nd BImSchV scope	<DL	<DL
2271	42nd BImSchV scope	1030	1300
2272	42nd BImSchV scope	800	1500
2273	42nd BImSchV scope	<DL	<DL
2274	42nd BImSchV scope	<DL	<DL
2275	42nd BImSchV scope	<DL	250
2276	42nd BImSchV scope	2100	60
2277	42nd BImSchV scope	<DL	<DL
2278	42nd BImSchV scope	<DL	<DL
2279	other industrial site	35000	44000
2280	other industrial site	37000	59000
2281	other industrial site	1700	700
2282	other industrial site	19000	17000
2283	42nd BImSchV scope	1800	108000
2284	42nd BImSchV scope	<DL	<DL
2285	42nd BImSchV scope	<DL	<DL
2286	42nd BImSchV scope	<DL	30
2287	42nd BImSchV scope	1300	7800
2288	42nd BImSchV scope	65000	31000
2289	42nd BImSchV scope	25000	30000
2290	42nd BImSchV scope	30	<DL
2291	42nd BImSchV scope	1700	310

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2292	42nd BImSchV scope	10	20
2293	42nd BImSchV scope	10	<DL
2294	42nd BImSchV scope	380	180
2295	42nd BImSchV scope	44000	2300
2296	42nd BImSchV scope	13000	18000
2297	42nd BImSchV scope	12000	22000
2298	42nd BImSchV scope	1500	4100
2299	42nd BImSchV scope	240	520
2300	42nd BImSchV scope	70	20
2301	other industrial site	180000	29000
2302	42nd BImSchV scope	50	550
2303	42nd BImSchV scope	20	130
2304	42nd BImSchV scope	9000	9000
2305	42nd BImSchV scope	380000	540000
2306	42nd BImSchV scope	5700	770
2307	42nd BImSchV scope	290	1400
2308	42nd BImSchV scope	6200	3100
2309	42nd BImSchV scope	36000	31000
2310	42nd BImSchV scope	15000	21000
2311	42nd BImSchV scope	<DL	<DL
2312	42nd BImSchV scope	540	600
2313	42nd BImSchV scope	210	180
2314	42nd BImSchV scope	5000	2900
2315	42nd BImSchV scope	190	790
2316	42nd BImSchV scope	500	na
2317	42nd BImSchV scope	720	410
2318	42nd BImSchV scope	20	1300
2319	42nd BImSchV scope	270000	190000
2320	42nd BImSchV scope	<DL	<DL
2321	42nd BImSchV scope	29000	70000
2322	42nd BImSchV scope	210	440
2323	42nd BImSchV scope	60	170
2324	42nd BImSchV scope	1500	6000
2325	other industrial site	380000	240000
2326	other industrial site	20	30
2327	other industrial site	32400	47400
2328	42nd BImSchV scope	16000	15000
2329	42nd BImSchV scope	17000	2600
2330	42nd BImSchV scope	320000	TNTC
2331	42nd BImSchV scope	590	130
2332	42nd BImSchV scope	220000	TNTC
2333	42nd BImSchV scope	3600	1300
2334	42nd BImSchV scope	21000	49000
2335	42nd BImSchV scope	460	10
2336	42nd BImSchV scope	<DL	<DL

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2337	42nd BImSchV scope	1600	13000
2338	other industrial site	7000	11000
2339	42nd BImSchV scope	2900	7200
2340	42nd BImSchV scope	740	20
2341	42nd BImSchV scope	1020	1000
2342	42nd BImSchV scope	90	110
2343	42nd BImSchV scope	120	180
2344	42nd BImSchV scope	500	330
2345	42nd BImSchV scope	19	60
2346	42nd BImSchV scope	120	280
2347	42nd BImSchV scope	30	20
2348	42nd BImSchV scope	<DL	1700
2349	42nd BImSchV scope	300	340
2350	42nd BImSchV scope	380	80
2351	42nd BImSchV scope	80	10
2352	42nd BImSchV scope	<DL	10
2353	42nd BImSchV scope	430	580
2354	42nd BImSchV scope	500	230
2355	42nd BImSchV scope	2000	1390
2356	42nd BImSchV scope	370	1300
2357	42nd BImSchV scope	40	100
2358	42nd BImSchV scope	150	30
2359	42nd BImSchV scope	710	690
2360	42nd BImSchV scope	590	810
2361	42nd BImSchV scope	3600	3200
2362	42nd BImSchV scope	240	300
2363	42nd BImSchV scope	4200	280
2364	42nd BImSchV scope	75600	12000
2365	42nd BImSchV scope	<DL	<DL
2366	42nd BImSchV scope	<DL	<DL
2367	42nd BImSchV scope	<DL	<DL
2368	42nd BImSchV scope	<DL	800
2369	42nd BImSchV scope	160	10
2370	other industrial site	660	1800
2371	other industrial site	<DL	<DL
2372	other industrial site	110	200
2373	other industrial site	<DL	20
2374	other industrial site	<DL	<DL
2375	other industrial site	<DL	<DL
2376	42nd BImSchV scope	1100	90
2377	42nd BImSchV scope	240	240
2378	42nd BImSchV scope	189000	162000
2379	42nd BImSchV scope	129600	54000
2380	other industrial site	237600	118800
2381	42nd BImSchV scope	680	260

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2382	42nd BImSchV scope	<DL	<DL
2383	42nd BImSchV scope	390	40
2384	42nd BImSchV scope	12000	16800
2385	42nd BImSchV scope	50	<DL
2386	42nd BImSchV scope	10	<DL
2387	42nd BImSchV scope	<DL	10
2388	42nd BImSchV scope	<DL	<DL
2389	42nd BImSchV scope	<DL	19
2390	42nd BImSchV scope	<DL	<DL
2391	42nd BImSchV scope	<DL	<DL
2392	42nd BImSchV scope	<DL	<DL
2393	42nd BImSchV scope	90	<DL
2394	42nd BImSchV scope	3300	2300
2395	42nd BImSchV scope	8700	8200
2396	42nd BImSchV scope	400	330
2397	42nd BImSchV scope	18000	22200
2398	42nd BImSchV scope	170	80
2399	42nd BImSchV scope	580	550
2400	other industrial site	22200	33600
2401	other industrial site	930	12600
2402	other industrial site	10	10
2403	other industrial site	3700	8400
2404	other industrial site	57000	54000
2405	other industrial site	151200	172800
2406	other industrial site	<DL	<DL
2407	other industrial site	170	120
2408	other industrial site	2700	1500
2409	42nd BImSchV scope	170	180
2410	42nd BImSchV scope	5300	30000
2411	42nd BImSchV scope	740	50
2412	42nd BImSchV scope	120	4600
2413	42nd BImSchV scope	43000	5700
2414	42nd BImSchV scope	880	250
2415	42nd BImSchV scope	38000	370
2416	42nd BImSchV scope	580000	800
2417	42nd BImSchV scope	100000	230
2418	42nd BImSchV scope	600000	9700
2419	42nd BImSchV scope	60	90
2420	42nd BImSchV scope	180	<DL
2421	42nd BImSchV scope	830	120
2422	42nd BImSchV scope	910	130
2423	42nd BImSchV scope	160	120
2424	42nd BImSchV scope	2200	160
2425	42nd BImSchV scope	3100	4000
2426	42nd BImSchV scope	2600	1300

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2427	42nd BImSchV scope	16000	5600
2428	other industrial site	22000	23000
2429	42nd BImSchV scope	<DL	30
2430	42nd BImSchV scope	3100	9600
2431	42nd BImSchV scope	340	1500
2432	42nd BImSchV scope	260	1600
2433	42nd BImSchV scope	560	TNTC
2434	other industrial site	350000	210000
2435	42nd BImSchV scope	16200	21600
2436	other industrial site	16200	27000
2437	42nd BImSchV scope	16000	11000
2438	42nd BImSchV scope	49000	16000
2439	42nd BImSchV scope	16000	11000
2440	42nd BImSchV scope	<DL	60
2441	42nd BImSchV scope	440	40
2442	42nd BImSchV scope	10	10
2443	42nd BImSchV scope	<DL	10
2444	42nd BImSchV scope	440	100
2445	42nd BImSchV scope	2200	3800
2446	42nd BImSchV scope	2600	4300
2447	42nd BImSchV scope	19000	16000
2448	42nd BImSchV scope	<DL	<DL
2449	42nd BImSchV scope	1400	1300
2450	42nd BImSchV scope	2000	3300
2451	42nd BImSchV scope	4500	9600
2452	42nd BImSchV scope	3300	7200
2453	42nd BImSchV scope	4900	5400
2454	42nd BImSchV scope	97000	410000
2455	42nd BImSchV scope	<DL	<DL
2456	42nd BImSchV scope	100	430
2457	42nd BImSchV scope	720	960
2458	42nd BImSchV scope	290	80
2459	42nd BImSchV scope	1200	2200
2460	42nd BImSchV scope	<DL	<DL
2461	42nd BImSchV scope	<DL	30
2462	42nd BImSchV scope	10	190
2463	42nd BImSchV scope	450	1500
2464	42nd BImSchV scope	90	160
2465	42nd BImSchV scope	1600	670
2466	42nd BImSchV scope	12600	24000
2467	other industrial site	97200	108000
2468	42nd BImSchV scope	20	50
2469	42nd BImSchV scope	1700	1600
2470	42nd BImSchV scope	2200	1400
2471	42nd BImSchV scope	210	140

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2472	42nd BImSchV scope	190	3400
2473	42nd BImSchV scope	<DL	<DL
2474	42nd BImSchV scope	720	310
2475	42nd BImSchV scope	100	110
2476	42nd BImSchV scope	180	140
2477	42nd BImSchV scope	740	na
2478	42nd BImSchV scope	120	180
2479	42nd BImSchV scope	20	40
2480	42nd BImSchV scope	10	6000
2481	42nd BImSchV scope	<DL	<DL
2482	42nd BImSchV scope	300	na
2483	42nd BImSchV scope	930	<DL
2484	42nd BImSchV scope	130000	3000
2485	42nd BImSchV scope	310	260
2486	42nd BImSchV scope	230	80
2487	42nd BImSchV scope	120	80
2488	42nd BImSchV scope	210	200
2489	42nd BImSchV scope	780	560
2490	42nd BImSchV scope	10	<DL
2491	42nd BImSchV scope	40	10
2492	42nd BImSchV scope	20	30
2493	42nd BImSchV scope	1500	540
2494	42nd BImSchV scope	1020	370
2495	42nd BImSchV scope	960	410
2496	42nd BImSchV scope	4000	3000
2497	42nd BImSchV scope	80	90
2498	42nd BImSchV scope	48600	37800
2499	42nd BImSchV scope	110	3000
2500	42nd BImSchV scope	<DL	<DL
2501	42nd BImSchV scope	<DL	<DL
2502	42nd BImSchV scope	<DL	<DL
2503	42nd BImSchV scope	1600	2400
2504	42nd BImSchV scope	500	200
2505	42nd BImSchV scope	25200	16800
2506	42nd BImSchV scope	9000	13200
2507	42nd BImSchV scope	2900	15000
2508	42nd BImSchV scope	162000	486000
2509	42nd BImSchV scope	690	<DL
2510	42nd BImSchV scope	243000	TNTC
2511	other industrial site	11000	13000
2512	42nd BImSchV scope	10800	2400
2513	42nd BImSchV scope	10800	210600
2514	42nd BImSchV scope	16000	120
2515	42nd BImSchV scope	290	30
2516	42nd BImSchV scope	<DL	270

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2517	42nd BImSchV scope	<DL	90
2518	42nd BImSchV scope	<DL	<DL
2519	42nd BImSchV scope	<DL	10
2520	42nd BImSchV scope	<DL	80
2521	42nd BImSchV scope	<DL	30
2522	42nd BImSchV scope	70	20
2523	other industrial site	30	<DL
2524	42nd BImSchV scope	240	210
2525	42nd BImSchV scope	16200	9600
2526	42nd BImSchV scope	<DL	<DL
2527	42nd BImSchV scope	11000	510
2528	42nd BImSchV scope	11000	30
2529	42nd BImSchV scope	30	<DL
2530	42nd BImSchV scope	<DL	<DL
2531	42nd BImSchV scope	<DL	30
2532	other industrial site	16200	21600
2533	42nd BImSchV scope	15000	3100
2534	42nd BImSchV scope	3300	4500
2535	42nd BImSchV scope	13000	3600
2536	42nd BImSchV scope	1800	2100
2537	42nd BImSchV scope	29000	34000
2538	42nd BImSchV scope	<DL	420
2539	42nd BImSchV scope	250	190
2540	42nd BImSchV scope	34000	13000
2541	42nd BImSchV scope	56000	23000
2542	42nd BImSchV scope	390	160
2543	42nd BImSchV scope	81000	92000
2544	42nd BImSchV scope	2300	17000
2545	42nd BImSchV scope	1900	14000
2546	42nd BImSchV scope	80	30
2547	other industrial site	75000	92000
2548	42nd BImSchV scope	490	650
2549	42nd BImSchV scope	40	100
2550	42nd BImSchV scope	280	360
2551	42nd BImSchV scope	70	140
2552	42nd BImSchV scope	1200	370
2553	42nd BImSchV scope	2400	730
2554	42nd BImSchV scope	<DL	<DL
2555	42nd BImSchV scope	1300	3200
2556	42nd BImSchV scope	190	530
2557	42nd BImSchV scope	800	1200
2558	42nd BImSchV scope	2300	2900
2559	42nd BImSchV scope	1230	2200
2560	42nd BImSchV scope	100	140
2561	42nd BImSchV scope	19000	150000

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2562	42nd BImSchV scope	17000	150000
2563	other industrial site	170000	16000
2564	other industrial site	40000	44000
2565	other industrial site	<DL	<DL
2566	other industrial site	1700	4900
2567	other industrial site	530	40
2568	other industrial site	230000	320000
2569	other industrial site	270	310
2570	other industrial site	60	430
2571	42nd BImSchV scope	40	230
2572	42nd BImSchV scope	16000	6600
2573	42nd BImSchV scope	10	<DL
2574	42nd BImSchV scope	15000	118800
2575	other industrial site	<DL	<DL
2576	other industrial site	<DL	<DL
2577	other industrial site	43200	14400
2578	other industrial site	13000	10000
2579	42nd BImSchV scope	10	<DL
2580	42nd BImSchV scope	180	740
2581	42nd BImSchV scope	16200	16200
2582	42nd BImSchV scope	17000	17000
2583	42nd BImSchV scope	86400	172800
2584	42nd BImSchV scope	150	50
2585	42nd BImSchV scope	2000	4100
2586	42nd BImSchV scope	4800	10800
2587	42nd BImSchV scope	15300	16200
2588	42nd BImSchV scope	20	<DL
2589	other industrial site	3700	2600
2590	42nd BImSchV scope	380	130
2591	42nd BImSchV scope	<DL	<DL
2592	42nd BImSchV scope	194400	113400
2593	42nd BImSchV scope	118800	64800
2594	42nd BImSchV scope	<DL	10
2595	42nd BImSchV scope	<DL	<DL
2596	42nd BImSchV scope	<DL	<DL
2597	42nd BImSchV scope	1300	360
2598	42nd BImSchV scope	9300	3600
2599	42nd BImSchV scope	9600	3700
2600	42nd BImSchV scope	113400	183600
2601	42nd BImSchV scope	<DL	<DL
2602	42nd BImSchV scope	32400	58800
2603	42nd BImSchV scope	3000	2000
2604	42nd BImSchV scope	1900	420
2605	42nd BImSchV scope	8200	17400
2606	42nd BImSchV scope	102600	140400

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2607	42nd BImSchV scope	250	10
2608	42nd BImSchV scope	16200	18000
2609	other industrial site	32400	26400
2610	42nd BImSchV scope	620	2800
2611	42nd BImSchV scope	90	40
2612	42nd BImSchV scope	730	710
2613	42nd BImSchV scope	380	130
2614	42nd BImSchV scope	200	90
2615	42nd BImSchV scope	850	na
2616	42nd BImSchV scope	410	120
2617	42nd BImSchV scope	<DL	<DL
2618	42nd BImSchV scope	22200	4500
2619	42nd BImSchV scope	50	30
2620	42nd BImSchV scope	150	na
2621	42nd BImSchV scope	490	260
2622	42nd BImSchV scope	140	40
2623	42nd BImSchV scope	480	1700
2624	42nd BImSchV scope	110	160
2625	42nd BImSchV scope	40	na
2626	42nd BImSchV scope	460	720
2627	42nd BImSchV scope	530	500
2628	42nd BImSchV scope	370	na
2629	42nd BImSchV scope	800	120
2630	42nd BImSchV scope	510	1420
2631	42nd BImSchV scope	90	820
2632	42nd BImSchV scope	1500	860
2633	42nd BImSchV scope	100	260
2634	other industrial site	10800	10800
2635	other industrial site	28800	20100
2636	other industrial site	19800	13100
2637	other industrial site	12100	10500
2638	other industrial site	13200	6000
2639	other industrial site	12000	18000
2640	other industrial site	<DL	<DL
2641	other industrial site	10	<DL
2642	other industrial site	<DL	<DL
2643	other industrial site	<DL	<DL
2644	other industrial site	10	<DL
2645	other industrial site	<DL	<DL
2646	42nd BImSchV scope	360	2100
2647	42nd BImSchV scope	410	1060
2648	42nd BImSchV scope	170	130
2649	42nd BImSchV scope	460	760
2650	42nd BImSchV scope	590	540
2651	42nd BImSchV scope	160	260

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2652	42nd BImSchV scope	350	2000
2653	42nd BImSchV scope	460	1180
2654	42nd BImSchV scope	430	860
2655	42nd BImSchV scope	240	na
2656	other industrial site	200	150
2657	other industrial site	360	710
2658	42nd BImSchV scope	2800	740
2659	42nd BImSchV scope	120	870
2660	42nd BImSchV scope	<DL	30
2661	42nd BImSchV scope	<DL	<DL
2662	42nd BImSchV scope	<DL	<DL
2663	42nd BImSchV scope	60	80
2664	42nd BImSchV scope	230	210
2665	42nd BImSchV scope	10800	1800
2666	42nd BImSchV scope	4800	3200
2667	42nd BImSchV scope	<DL	<DL
2668	42nd BImSchV scope	16200	16200
2669	42nd BImSchV scope	16200	10800
2670	42nd BImSchV scope	1800	2900
2671	42nd BImSchV scope	3600	3400
2672	42nd BImSchV scope	4200	4400
2673	42nd BImSchV scope	970	980
2674	42nd BImSchV scope	<DL	10
2675	42nd BImSchV scope	<DL	<DL
2676	42nd BImSchV scope	220	10
2677	42nd BImSchV scope	1100	1690
2678	42nd BImSchV scope	<DL	<DL
2679	42nd BImSchV scope	1480	1300
2680	42nd BImSchV scope	3500	TNTC
2681	42nd BImSchV scope	<DL	<DL
2682	42nd BImSchV scope	10	<DL
2683	42nd BImSchV scope	2200	10800
2684	42nd BImSchV scope	<DL	10
2685	other industrial site	1800	27000
2686	other industrial site	<DL	340
2687	other industrial site	<DL	<DL
2688	other industrial site	70	1580
2689	42nd BImSchV scope	30000	75600
2690	other industrial site	280000	135000
2691	42nd BImSchV scope	1400	2200
2692	42nd BImSchV scope	270	290
2693	42nd BImSchV scope	1700	16200
2694	42nd BImSchV scope	32400	37800
2695	42nd BImSchV scope	<DL	10800
2696	42nd BImSchV scope	151200	97200

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2697	42nd BImSchV scope	<DL	<DL
2698	42nd BImSchV scope	<DL	<DL
2699	42nd BImSchV scope	3300	3600
2700	42nd BImSchV scope	TNTC	TNTC
2701	42nd BImSchV scope	2100	2400
2702	42nd BImSchV scope	1800	1600
2703	42nd BImSchV scope	194400	167400
2704	42nd BImSchV scope	86400	140400
2705	other industrial site	199800	118800
2706	42nd BImSchV scope	<DL	360
2707	42nd BImSchV scope	10	100
2708	42nd BImSchV scope	60	140
2709	42nd BImSchV scope	220	270
2710	42nd BImSchV scope	5200	4800
2711	other industrial site	475200	124200
2712	other industrial site	97200	70200
2713	42nd BImSchV scope	240	113400
2714	other industrial site	160	97200
2715	42nd BImSchV scope	2900	2800
2716	42nd BImSchV scope	1230	2500
2717	42nd BImSchV scope	230	270
2718	42nd BImSchV scope	140400	102600
2719	42nd BImSchV scope	178200	151200
2720	42nd BImSchV scope	64800	81000
2721	42nd BImSchV scope	81000	140400
2722	42nd BImSchV scope	10800	6000
2723	42nd BImSchV scope	11400	3400
2724	42nd BImSchV scope	4500	1900
2725	42nd BImSchV scope	172800	16200
2726	42nd BImSchV scope	10	20
2727	42nd BImSchV scope	345600	32400
2728	42nd BImSchV scope	2400	760
2729	42nd BImSchV scope	1400	1660
2730	42nd BImSchV scope	86400	129600
2731	42nd BImSchV scope	6800	453600
2732	other industrial site	9200	280800
2733	42nd BImSchV scope	10200	10800
2734	42nd BImSchV scope	220	140
2735	42nd BImSchV scope	670	640
2736	42nd BImSchV scope	760	1190
2737	42nd BImSchV scope	300	380
2738	42nd BImSchV scope	890	TNTC
2739	42nd BImSchV scope	910	1800
2740	42nd BImSchV scope	130	90
2741	42nd BImSchV scope	3700	3200

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2742	42nd BImSchV scope	110	50
2743	42nd BImSchV scope	730	TNTC
2744	42nd BImSchV scope	1000	540
2745	42nd BImSchV scope	540	210
2746	42nd BImSchV scope	68400	86400
2747	42nd BImSchV scope	TNTC	453600
2748	42nd BImSchV scope	620	440
2749	42nd BImSchV scope	430	1340
2750	42nd BImSchV scope	1630	1800
2751	42nd BImSchV scope	630	180
2752	42nd BImSchV scope	110	50
2753	42nd BImSchV scope	5200	5400
2754	42nd BImSchV scope	1390	2500
2755	42nd BImSchV scope	540000	TNTC
2756	42nd BImSchV scope	<DL	40
2757	42nd BImSchV scope	<DL	<DL
2758	42nd BImSchV scope	380	870
2759	42nd BImSchV scope	110	70
2760	42nd BImSchV scope	<DL	60
2761	42nd BImSchV scope	<DL	<DL
2762	42nd BImSchV scope	3300	1200
2763	42nd BImSchV scope	167400	135000
2764	42nd BImSchV scope	21600	20400
2765	42nd BImSchV scope	2500	4600
2766	42nd BImSchV scope	2600	5400
2767	42nd BImSchV scope	<DL	<DL
2768	42nd BImSchV scope	10	<DL
2769	42nd BImSchV scope	<DL	10
2770	42nd BImSchV scope	1500	1640
2771	42nd BImSchV scope	10800	124200
2772	42nd BImSchV scope	259200	TNTC
2773	42nd BImSchV scope	2300	35
2774	42nd BImSchV scope	367200	TNTC
2775	other industrial site	21600	156600
2776	42nd BImSchV scope	680	280
2777	42nd BImSchV scope	32400	16200
2778	42nd BImSchV scope	<DL	10
2779	42nd BImSchV scope	<DL	40
2780	42nd BImSchV scope	<DL	60
2781	42nd BImSchV scope	140	130
2782	42nd BImSchV scope	<DL	<DL
2783	42nd BImSchV scope	16200	27000
2784	42nd BImSchV scope	92880	172800
2785	42nd BImSchV scope	240	170
2786	42nd BImSchV scope	10	<DL

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2787	42nd BImSchV scope	<DL	<DL
2788	42nd BImSchV scope	290	30
2789	42nd BImSchV scope	10800	2600
2790	42nd BImSchV scope	10800	2200
2791	42nd BImSchV scope	<DL	<DL
2792	42nd BImSchV scope	27000	21600
2793	42nd BImSchV scope	5400	3600
2794	42nd BImSchV scope	27000	16200
2795	42nd BImSchV scope	16200	16200
2796	42nd BImSchV scope	5400	3000
2797	42nd BImSchV scope	1060	156600
2798	42nd BImSchV scope	2600	3000
2799	42nd BImSchV scope	194400	221400
2800	42nd BImSchV scope	29000	16200
2801	42nd BImSchV scope	86400	70200
2802	42nd BImSchV scope	10800	3200
2803	42nd BImSchV scope	3900	10800
2804	42nd BImSchV scope	21600	64800
2805	other industrial site	10800	16200
2806	other industrial site	<DL	20
2807	other industrial site	<DL	10
2808	other industrial site	<DL	<DL
2809	other industrial site	3800	3000
2810	other industrial site	<DL	1600
2811	other industrial site	4300	16200
2812	other industrial site	2500	6800
2813	other industrial site	1040	2300
2814	other industrial site	<DL	<DL
2815	other industrial site	570	129600
2816	42nd BImSchV scope	6100	5400
2817	other industrial site	240	310
2818	42nd BImSchV scope	140	170
2819	42nd BImSchV scope	280	760
2820	42nd BImSchV scope	560	150
2821	42nd BImSchV scope	430	760
2822	42nd BImSchV scope	151200	113400
2823	42nd BImSchV scope	<DL	<DL
2824	42nd BImSchV scope	27000	240
2825	42nd BImSchV scope	10800	40
2826	42nd BImSchV scope	21600	<DL
2827	42nd BImSchV scope	240	190
2828	42nd BImSchV scope	43200	27000
2829	42nd BImSchV scope	5400	5400
2830	42nd BImSchV scope	2800	5400
2831	42nd BImSchV scope	80	290

Sample #	Water type	HPC 22 °C [cfu/mL]	HPC 36 °C [cfu/mL]
2832	other industrial site	178200	75600
2833	42nd BImSchV scope	<DL	<DL
2834	42nd BImSchV scope	5600	10800
2835	42nd BImSchV scope	90	270
2836	42nd BImSchV scope	<DL	50
2837	42nd BImSchV scope	162000	580
2838	42nd BImSchV scope	24600	48600
2839	42nd BImSchV scope	310	290
2840	other industrial site	TNTC	302400
2841	other industrial site	8400	1120
2842	42nd BImSchV scope	330	100
2843	42nd BImSchV scope	100	237600
2844	42nd BImSchV scope	<DL	<DL
2845	42nd BImSchV scope	48600	118800
2846	other industrial site	<DL	<DL
2847	other industrial site	660	275400
2848	42nd BImSchV scope	205200	199800
2849	42nd BImSchV scope	30	<DL
2850	42nd BImSchV scope	86400	118800
2851	42nd BImSchV scope	10800	8600
2852	42nd BImSchV scope	194400	156600
2853	42nd BImSchV scope	870	2400
2854	42nd BImSchV scope	960	1400
2855	42nd BImSchV scope	64800	64800
2856	42nd BImSchV scope	820	324000
2857	42nd BImSchV scope	151200	216000
2858	42nd BImSchV scope	560	440
2859	42nd BImSchV scope	570	1700
2860	42nd BImSchV scope	48600	194400
2861	42nd BImSchV scope	<DL	10
2862	42nd BImSchV scope	180	360
2863	42nd BImSchV scope	583200	TNTC
2864	42nd BImSchV scope	1070	770
2865	42nd BImSchV scope	380	324000
2866	42nd BImSchV scope	40	90
2867	42nd BImSchV scope	2200	860
2868	other industrial site	680	129600

Danksagung / Acknowledgement

Herrn Prof. Dr. Dr. Martin Exner spreche ich allergrößten Dank aus, dass er mir die Möglichkeit bot, diese Arbeit am Institut für Hygiene und Öffentliche Gesundheit anzufertigen und die Erstbegutachtung übernommen hat.

Herrn Prof. Dr. Uwe Deppenmeier danke ich vielmals für die Zweitbegutachtung.

Herrn Prof. Dr. Michael Hofmann und Frau Prof. Dr. Ursel Heudorf danke ich sehr für Ihre Begutachtung als fachnahes und fachfremdes Mitglied der Prüfungskommission.

Vielmals danke ich Herrn Dr. Stefan Pleischl für seine Betreuung, seine Hilfe in fachlichen und praktischen Fragen, die Vermittlung von Kontakten und seine Unterstützung bei der Fertigstellung dieser Arbeit.

Großen Dank möchte ich Herrn Dr. Christoph Koch für die Beantwortung und Hilfe bei zahlreichen fachlichen und Computer relevanten Fragen aussprechen.

I would like to thank the company IDEXX for the possibility to carry out the comparative studies. In particular, I would like to thank Manja Blazer, who taught me a lot. I would like to thank Darron Steggles, Brian Swalla and Dan Broder for their helpfulness.

Auch der Firma Evonik danke ich ausdrücklich für die Möglichkeit, Studien an den Kühltürmen durchzuführen. Ganz besonderer Dank geht an alle Personen der Abteilung, die die Kühltürme überwachen und mich bei der Durchführung der Experimente unterstützt und begleitet, Daten zur Verfügung gestellt, den Plattenhalter gebaut, meine vielen Fragen zum Kühlturm-Management beantwortet haben und bei den Plattenentnahmen zur Hand gingen.

Allen Mitarbeitern des IHPH, die mich bei der Erstellung dieser Arbeit unterstützt haben, insbesondere Birgit Göing, Frank Brändle und Nicole Zacharias danke ich sehr.

Meinen Eltern bin ich sehr dankbar, dass sie mich stets unterstützt haben, zu promovieren und mir in allen Belangen beigestanden haben.

Für seine Unterstützung, Rücksicht und Ermutigung danke ich meinem Mann von Herzen.

Auch meinen Freunden möchte ich für Ihre Motivation und Hilfsbereitschaft danken.

Nicht zu vergessen ist Sugar, der mich stets motiviert hat, das Ziel nicht aus den Augen zu verlieren.