World Health Organization

WHO recommendations on scientific, analytical and epidemiological developments relevant to the parameters for bathing water quality in the Bathing Water Directive (2006/7/EC)

Recommendations

FINAL REPORT

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Disclaimer

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Summary statement

The main objective of Directive 2006/7/EC of the European Parliament and of the Council concerning the management of bathing water quality (hereafter 'the Bathing Water Directive') is the protection of public health. In order to achieve this aim it is important to periodically review the parameters and methodology used for ascribing bathing water quality.

Research into bathing water has continued since the publication of the WHO Guidelines in 2003 and the current Bathing Water Directive. This document summarises, in a series of fact sheets, the recent scientific literature on the existing Bathing Water Directive parameters (intestinal enterococci and *E. coli*). It also examines the feasibility of possible additional parameters (viral indicator(s) and harmful algal blooms) and considers wider/emerging issues as a series of fact sheets.

In addition to a synthesis of the literature and an examination of the current classification system, it also includes inputs from Member States' representatives through completion of a bathing water questionnaire, expert and stakeholder group meetings[1] and feedback received[2] on background documents including the draft fact sheets. It represents the recommendations of the World Health Organization on updating Annex I of the Bathing Water Directive.

In synopsis, it is recommended that the two current parameters (intestinal enterococci and *E. coli*) as well as the four levels within the current classification system (excellent, good, sufficient and poor) should be retained within the Bathing Water Directive.

The proposed changes include the increase of the annual minimum number of samples from four to twenty and the usage of 95-percentile value for each category of the classification system instead of a mixture of 95- and 90-percentile water quality standards. To reduce the misclassification of sites, where the data is not shown to be $\log_{10}$ normally distributed (using the Shapiro-Wilks test), the use of the Hazen method is recommended instead of the Annex II percentile calculation. Further, the ISO method (9308-1) for *E. coli* analysis is no longer recommended as following its update it is suitable for waters with low bacterial numbers and, as such not applicable to bathing waters.

The discussion on the feasibility of possible additional parameters, has led to a conclusion that evidence does not support inclusion of a viral indicator or pathogen as a regulatory parameter at this time.

Simultaneously, the current system (i.e. consideration as part of the bathing water profile) for marine phytoplankton has been found fit for purpose.

[1] 30-31st March 2017, Berlin, Meeting on WHO technical advice to the European Bathing Water Directive Annex I;
5th October 2017, Brussels, Meeting of the EC informal experts group on the implementation of Directive 2006/7/EC (Bathing Water Directive);
24th November 2017, Brussels, Stakeholder consultation on WHO recommendations relevant to the parameters for bathing water quality in the BWD;
24-25th January 2018, Geneva, WHO Expert Group meeting on recreational water quality
[2] 22nd September 2017, Ispra, European Microbiology Expert Group meeting

It is advised that locations at risk of freshwater cyanobacterial blooms should be subject to a new classification/management system based on guidance levels currently under development by the World Health Organization and should allow Member States to choose which parameters to monitor (biovolume, chlorophyll-a, phycocyanin, transparency, toxin concentration).

On five wider emerging issues identified, it is suggested that the bathing profiles of the locations where either swimmer's itch or wound infections caused by, for example, *Vibrio* spp. are likely or known to have occurred, should provide members of the public with the necessary information as well as advice on bather hygiene measures.

Surveillance of environmental water for antimicrobial resistance is in a research phase and therefore not ready for regulatory use.

Ongoing research on the issue of microplastics that falls within the scope of the Marine Strategy Framework Directive should reveal in the short to medium term whether it is relevant also for the Bathing Water Directive.

The potential application of the Bathing Water Directive, currently restricted to bathers, to other recreational activities might need to be considered in future, especially if the non-bathing use of sites continues to increase. It has been concluded however, that a wide variety of recreational water activities may take place at bathing water locations but to specifically take account of these activities would potentially require different and additional sampling locations, an extended sampling period (as some activities take place outside of the traditional bathing season) and a possible zoning of the bathing area.

More details and background to these recommendations and key messages are provided in Chapter 3. The full conclusions, explanation and scientific justification are included in the individual fact sheets (A-E).

Contents

List of abbreviations and acronyms

AdV	adenovirus
AFRI	acute febrile respiratory illness
AMR	antimicrobial resistance
ANN	artificial neural networks
AOR	adjusted odds ratio
ASP	amnesic shellfish poisoning
ATX	anatoxins
BAdV	bovine adenovirus
BAV	beach action value
BPyV	bovine polyomavirus
BV	biovolume
BWD	Bathing Water Directive
CB	cyanobacteria
CCE	calibrator cell equivalents
CFP	ciguatera fish poisoning
cfu	colony forming unit
Chl-a	chlorophyll-a
CI	confidence interval
CYN	cylindrospermopsin
E. coli	*Escherichia coli*
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
ELISA	enzyme-linked immunosorbent assay
ENT	enterococci
EU	European Union
EV	enterovirus
FIO	faecal indicator organism
GC	genome copies
GI	gastrointestinal
GM	geometric mean
HAB	harmful algal bloom
HAEDAT	harmful algal events dataset
HCGI	highly credible gastrointestinal illness
HPLC	high performance liquid chromatography
ISO	International Organization for Standardization
LoD	limits of determination
MC	microcystins
MLR	multiple linear regression
MPN	most probable number
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MSFD	Marine Strategy Framework Directive
MST	microbial source tracking
NOD	nodularin
NoV	norovirus
NoVGI	norovirus genogroup I
NoVGII	norovirus genogroup II
NSP	neurotoxic shellfish poisoning
OR	odds ratio
PAdV	porcine adenovirus

PC	phycocyanin
PCR	polymerase chain reaction
PDA	photodiode array
Pfu	plaque forming units
PPIA	protein phosphatase inhibition assay
PSP	paralytic shellfish poisoning
PyV	polyomavirus
QMRA	quantitative microbial risk assessment
QMSA	quantitative microbial source apportionment
qPCR	quantitative polymerase chain reaction
RR	relative risk
STX	saxitoxins
TP	total phosphorus
USEPA	United State Environmental Protection Agency
UV	ultraviolet
WHO	World Health Organization
WQTAG	water quality and health technical advisory group

1. Introduction

The European Commission (EC) is required to review the current Bathing Water Directive (BWD) – Directive 2006/7/EC[3], no later than 2020, "*with particular regard to the parameters for bathing water quality, including whether it would be appropriate to phase out the 'sufficient' classification or modify the applicable standards*". The EC is also required to "*have particular regard to World Health Organisation recommendations*" (Article 14).

In addition to the parameters currently included in the BWD (intestinal enterococci – ENT and *Escherichia coli – E. coli*) an initial screening process and expert consultation suggested that viral and harmful algal bloom (HAB) parameters should also be investigated for possible inclusion in a potential review of the Directive. The first three parameters provide an indication of faecal contamination, while the organisms that cause HABs are indigenous to the water (usually in low concentrations) and present a hazard to human health only when they occur in high concentrations (usually as a consequence of nutrient enrichment of the water – eutrophication).

As noted by the World Health Organization[4] (WHO), there are a number of ideal characteristics for a faecal microorganism to be considered as a regulatory parameter of public health significance for recreational waters. Thus, ideally, it should:

- "*have a health basis;*
- *have adequate information available to allow the derivation of guideline values (e.g. from epidemiological investigations);*
- *be sufficiently stable in water samples to allow meaningful results to be obtained from water quality analyses;*
- *have a standard method for analysis;*
- *be low cost to test;*
- *make low demands on staff training; and*
- *require basic equipment that is readily available.*"

The current version of the BWD diverges from the existing WHO Guidelines[3] in a number of ways, as summarised in Table 1 (further details on the different classification/water quality levels are given in Sections A2 and B2).

[3] OJ L 64, 4.3.2006, p. 37–51 (http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32006L0007)

[4] WHO (2003) Guidelines for safe recreational water environments. Volume 1: Coastal and fresh waters. World Health Organization, Geneva, Switzerland.

Table 1: Principal water quality parameter differences between the WHO Guidelines and BWD

Organization	Parameter	Water type	Water quality (/100ml)	Annual minimum sample no	Measure
WHO	ENT only	Fresh & marine	≤ 500[a]	20	95 percentile
EU	ENT	Fresh	≤ 330 [b]	4	90 percentile
	ENT	Marine	≤ 185 [b]	4	90 percentile
	E. coli	Fresh	≤ 900 [b]	4	90 percentile
	E. coli	Marine	≤ 500 [b]	4	90 percentile

[a] Based on the a rating of 'fair' (estimation of up to a 10% gastrointestinal illness risk)
[b] Based on 'sufficient' classification

This report represents the advice of the WHO, as a series of recommendations, for consideration in in a potential review of BWD. The EC may choose to deviate from these recommendations for policy purposes.

2. Methods

The parameters to be considered and approach (fact sheets) to be taken were discussed and decided upon at a WHO expert meeting in Berlin (March 2017). The parameter fact sheets are based on a focused review of the scientific literature (conducted by Lorna Fewtrell during 2017) to update the relevant information in the WHO 2003 Guidelines for Safe Recreational Water Environments[3] (Chapters 7 and 8) and the 2009 Addendum to the Guidelines[5] (Chapter 4) and inform WHO advice to the EC.

Input into the process was also received through a review of a background document by the European Microbiology Expert Group (EMEG) and the Joint Research Centre (JRC), two EC meetings (an informal Bathing Water Directive expert group meeting, 5/10/17 and a Stakeholder consultation meeting, 24/11/17), a questionnaire put to all Member States and feedback received on draft versions of the factsheets. During this process a number of emerging/wider issues were identified (primarily by Member States) which fell outside the remit of the parameter fact sheets. These areas have been covered, briefly, in an additional fact sheet (E: Emerging/wider issues).

The final recommendations were developed at a WHO water quality and health technical advisory group (WQTAG) meeting in January 2018.

[5] WHO (2009) Addendum to the WHO Guidelines for Safe Recreational Water Environments, Volume 1, Coastal and Fresh Waters. WHO/HSE/WSH/10.04. World Health Organization, Geneva, Switzerland.

3. Recommendations to the European Commission and key messages

The following sections are a ***summary*** of the recommendations and key messages with a brief explanation. The full details, including an explanation and scientific justification, are included in the individual fact sheets (A-E).

3.1 Current parameters

Recommendations

1. Intestinal enterococci and *E. coli* should be retained.
2. The four levels within the current classification system (excellent, good, sufficient and poor) should be retained.
3. The classification system for each category should be based on a 95-percentile value and not a mixture of 95- and 90-percentile water quality standards.
4. The annual minimum number of samples for an EU bathing water site should be increased to 20.
5. Data from bathing water sites (with at least 80 samples) should be tested for $\log_{10}$ normality. Where the data are shown to be $\log_{10}$ normally distributed, the calculation method in Annex II of the 2006 Bathing Water Directive should be used. Where the data do not exhibit $\log_{10}$ normality the Hazen calculation should be used. Where there are inadequate data available, it is suggested that the Hazen calculation is used.
6. The ISO method (9308-1) for *E. coli* analysis is no longer appropriate for the measurement of bathing water quality.
7. Sampling and sample analysis should be conducted by laboratories accredited for the methods being used.

Good practice

8. Bathing water quality should be representative of the whole bathing area. This should be confirmed by occasional spatial/beach shoreline transect sampling.
9. Temporal variability in water quality should be addressed by sampling at different times to characterise the bathing day in the overall compliance data set, or taking a precautionary approach and sampling when water quality is generally poorest.
10. Where predictive modelling is used to inform the public, the choice of model and methods of public information dissemination should be reported. The models should meet minimum requirements (including an explained variance of at least 50-60%) and the approach taken should be justifiable and auditable.
11. In a number of cases (such as microbial source tracking techniques and quantitative microbial risk assessment for use in bathing water profiling) it would be valuable to commission a detailed state of the art review, to provide standardised information and advice on their practical application to Member States.

Research

12. Research is needed on the minimum number of data points and the appropriate sampling strategy required for predictive model building and whether models could be used instead of a check sample to allow a return to use after a short-term pollution event.
13. Since the completion of European epidemiological research, additional microbiological methods have been developed (including those using quantitative polymerase chain reaction [qPCR] methods). If additional European-based epidemiological studies are conducted in the future, it is suggested that qPCR methods for ENT and *E. coli* are included

as part of the microbial water quality analysis to determine whether these tests could provide a good prediction of bathing water related illness.

3.1.1 Retention of current parameters

There is sound epidemiological evidence supporting the inclusion of ENT as a water quality parameter (see Section A3 and Table A2). Epidemiological studies are used to evaluate illness resulting from exposure to contaminants and/or activities and have been used to inform recreational water quality guidelines and regulations. There have been a number of studies conducted since the WHO Guidelines[6] were published, including significant work conducted in the European Union. These studies generally support the use of ENT especially at marine water sites, impacted by human faecal pollution.

The epidemiological evidence supporting the use of *E. coli* is not as strong as that for ENT, although there is some evidence that it may be a useful index of gastrointestinal illness in fresh water (see Section B3). It has also been shown to drive compliance with the BWD at some designated bathing water sites (often fresh water locations) and its continued use allows the examination of historical trends.

3.1.2 Retention of current classification system

The current classification system is based on indicator concentrations (ENT and *E. coli*) categorised as 'excellent', 'good' and 'sufficient', with water quality failing to achieve at least the sufficient level being considered to be 'poor' (see Sections A7 and B7). One of the requirements of the review was to consider if it would be appropriate to phase out the sufficient classification. Retention of the sufficient classification provides an incentive for progressive improvement and it is likely that if it were to be removed a number of beaches in this category would be de-designated, with probable negative effects on the local community and also a further reduction in water quality.

3.1.3 Classification based on 95 percentile values

The classification system (outlined above and in Table A1 and B1) currently uses both 95-percentile (excellent, good) and 90-percentile values (sufficient). This is confusing for the public and difficult to justify. It is recommended that all of the categories should be based on a 95-percentile value; this is in line with the WHO Guidelines approach and retains consistency (for the most part) with the existing BWD. Amending this will not change the level of health protection provided.

3.1.4 Minimum sample numbers

The sample number used in the classification calculation has a significant impact on the overall results. The current minimum sample number of 16 (based on an annual sample number of four) specified in the BWD leads to significant misclassification of the bathing water locations. The analysis (illustrated in Figures A1 and B1), conducted by WHO technical experts, suggests that bathing waters might be given the wrong classification in 15-20% of cases when only 16 samples are used; this would be reduced to less than 5% where 80 samples are used. Misclassification results in either adverse health effects experienced at a bathing water thought wrongly to be compliant or economic loss to local communities where a compliant water is reported to be 'Poor' (See Figure A1 and the

[6] WHO (2003) Guidelines for safe recreational water environments. Volume 1: Coastal and fresh waters. World Health Organization, Geneva, Switzerland.
WHO (2009) Addendum to the WHO Guidelines for Safe Recreational Water Environments, Volume 1, Coastal and Fresh Waters. WHO/HSE/WSH/10.04. World Health Organization, Geneva, Switzerland.

following text on page 20 below). In public health terms, using the epidemiological approach in WHO Guidelines for safe recreational water environments (2003), misclassification of a 'Poor' bathing water as 'Good' would imply an actual health risk of gastroenteritis exceeding 8.4% when the bathers should be assured of a health risk of 3-5% which could reduce confidence in the regulatory agencies concerned. It is, thus, recommended that the ***annual*** minimum sample number should be 20 per site, which means that classification in the European Union should be based on at least 80 samples taken over a four year period.

3.1.5 Test for log_{10} normality

Many biological parameters are thought to be log_{10} normally distributed (i.e. they assume a 'normal' distribution when transformed into log_{10} values). This is an assumption within the current BWD, as indicated by the prescribed percentile calculation method outlined in Annex II of the Directive. In a number of cases, however, bathing water quality datasets are not log_{10} normal (see Section A8). It is recommended, therefore, that data from bathing water sites with at least 80 samples should be tested for log_{10} normality (using the Shapiro-Wilk test). If log_{10} normality is demonstrated, the Annex II calculation can be used for percentile calculation. Where the data are not shown to be normally distributed, the Hazen calculation method should be used, as outlined in the WHO Guidelines addendum. This measure will reduce misclassification of sites.

3.1.6 ISO method 9308-1

Annex I of the BWD describes reference microbiological methods suitable for bathing water quality analysis. Since its publication, in 2006, one of the ISO methods cited for analysis of *E. coli* (9308-1) has been updated. It is now only suitable for waters with low bacterial numbers and, as such, the current version is not applicable to bathing waters (see Section B4).

3.1.7 Use of accredited laboratories

Accreditation determines the technical competence and integrity of organisations offering testing, inspection, calibration, verification and certification services. Bathing water quality sampling and microbiological analysis should, thus, only be conducted by laboratories accredited for the methods used. This should ensure that the sampling and analyses are done correctly and according to standard procedures.

3.1.8 Spatial water quality

At many recreational water sites the microbial water quality varies, often markedly, across the site. The BWD recommends that the monitoring point should be located either where most bathers are expected or where the greatest risk of pollution is anticipated. It is suggested that the suitability of the sampling point is confirmed by annual spatial/beach shoreline transect sampling.

3.1.9 Temporal water quality

Water quality at many locations is known to vary throughout the day (due to the influence of a number of factors including the intensity of sunlight and the presence of bathers). It is possible that if sampling is always done at the same time this variability will not be captured in the compliance data. This could be addressed by sampling at different times during the bathing season or, where this is not practical, a precautionary approach could be taken with sampling conducted when the water quality is likely to be poorest.

3.1.10 Predictive modelling

Where there are sufficient water quality data (often obtained through intensive, sampling programmes) it may be possible, through modelling, to predict the conditions when water quality will be poor (such as after rainfall). The use of such modelling programmes enables timely water quality data to be delivered to the public, to allow an informed choice to be made (Section A6 and B6). Where such predictive modelling is used, the model (which should be able to demonstrate explanatory power of at least 50-60% (R^2)) and predictive variables (e.g. rainfall) should be the choice of the Member State, but should be reported.

3.1.11 Provision of practical advice

There are a number of techniques, such as microbial source tracking (MST) and quantitative microbial risk assessment (QMRA), which are potentially valuable tools for ensuring that bathing water profiles accurately reflect the conditions of the bathing water (Section A5 and B5). While their use should be optional, it would be valuable to commission a detailed state of the art review on each of these areas to inform Member States on their practical application.

3.1.12 Research

When water quality samples are taken during short-term microbiological pollution events the results can currently be disregarded, provided a number of requirements are met, including the taking of an additional check sample at the end of the pollution period (Section A7 and B7). It may be that predictive modelling could be used instead of physical sampling to provide this check 'sample', although research is required in this area and also on the minimum number of data points required for model building.

New molecular methods for determining ENT and *E. coli* have been developed (Section A4 and B4). Currently their use is not widespread in Europe and there are no European studies which show their relationship with the health of bathers (Sections A3 and B3) so inclusion in the BWD is not recommended at this time. It is, however, suggested that they are incorporated as test water quality parameters into any future European epidemiological research.

3.2 Viral indicator

Recommendations

1. Current evidence does not support the inclusion of a viral indicator (or viral pathogen) as a regulatory parameter within the BWD.

Good practice and research

2. Viruses have a valuable role to play in microbial source tracking investigations and also quantitative microbial risk assessment, and it is suggested that these tools should be considered more widely in bathing water profiling
3. Research needs to include the identification of suitable candidate viral organisms and the development of standard methods suitable for bathing water use.

3.2.1 No viral parameter

Because of their role in recreational water illness and the recent development of new methods for analysis, there has been a suggestion that enteric viruses and/or bacteriophages (non-human pathogens which infect bacteria) could be used in water quality assessment (Section C1). There is,

however, currently insufficient evidence to support a regulatory role. Many of the candidate viruses examined to date are difficult to detect and are only present in very low levels in recreational water (Section C5). The acceptance and deployment of an analytical viral methodology is not widespread and there is a lack of standardized methods for bathing water (Section C6). In addition, there is currently insufficient epidemiological evidence to allow the derivation of regulatory levels (Section C4).

3.2.2 Microbial source tracking and quantitative microbial risk assessment tools

Microbial source tracking (MST) tools and quantitative microbial risk assessment (QMRA) are useful tools which could be used more widely in the bathing water profile process (Section A5). While they are not discussed specifically in the viral fact sheet, viruses have a major role to play in these tools with a number of the MST targets being genetic material derived from viruses (e.g. norovirus - see Table A3). It has also been suggested above (3.1.11) that it would be valuable to commission a detailed state of the art review on both MST and QMRA to inform Member States on their practical application.

3.2.3 Research needs

As a number of viruses clearly have a health basis, the suggestion for their regulatory inclusion is likely to remain. In order to progress, research needs to include identification of suitable candidate organisms (e.g. a human enteric virus with consistent shedding patterns) and the development of standard methods which are suitably sensitive and can be applied consistently across different laboratories; only then should additional European epidemiological studies be considered to examine possible dose-response relationships (Section C4).

3.3 Harmful algal blooms

Recommendations

1. Locations at risk of freshwater cyanobacterial blooms should be subject to a new classification/management system. This should be based on guidance levels currently under development by the World Health Organization and should allow Member States to choose which parameters to monitor (biovolume, chlorophyll-a, phycocyanin, transparency, toxin concentration).
2. A clear on-site indication (signage and public information) of cyanobacterial hazard should be given at those sites identified as being at risk.
3. The current system (i.e. consideration as part of the bathing water profile) for marine phytoplankton should be retained.

Good practice

4. Available cyanobacterial occurrence and proliferation models and research results should be collated and consolidated to provide guidance to Member States.

3.3.1 Freshwater cyanobacterial bloom classification/management system

From a recreational viewpoint, it is harmful algal blooms (HABs) caused by cyanobacteria (primarily, but not exclusively, in freshwaters) that are the main cause for concern in water bodies in the EU. Cyanobacteria are not, currently, assessed as part of the BWD assessment and classification, although there is a requirement to consider them as part of the bathing water profile (Section D1). It is recommended that locations that have been identified as at risk from freshwater cyanobacterial blooms should be subject to a new classification/management system. This should be separate from

the current classification system (based on ENT and *E. coli*), as the sources are different as are bloom behaviour and management responses. WHO guidance levels, for a variety of suitable parameters, are currently under development (Section D5 and D6). It is suggested that these are adopted and that Member States are allowed to choose which parameters to monitor based on the local experience and circumstances.

3.3.2 On-site signage

Sites subject to the recommended freshwater cyanobacterial classification/management system should display clear public information identifying them as 'at risk' (e.g. "prone to harmful blooms"). It is suggested that this is accompanied by a standard symbol. This information should be displayed even in the absence of a bloom. It is also suggested that clear guidance on how to recognise a bloom and what to do in such an event be displayed at the site, to allow for informed public choice.

3.3.3 Marine phytoplankton and the bathing water profile

The principal health concerns from marine HABs derive from the consumption of seafood which can concentrate toxins from a bloom (Section D1). On this basis, it is recommended that no change is needed to the current method used in the BWD for marine phytoplankton; i.e. their consideration as part of the bathing water profile. Where a bathing water profile identifies that there is a potential for proliferation, further investigations are required (including appropriate monitoring) to determine likely health risks. Where health risks are identified there is a requirement for adequate management measures which include public information.

3.3.4 Provision of advice

Some work has been conducted on the prediction of cyanobacterial occurrence and proliferation on the basis of water-body characteristics and history. Modelling cyanobacterial occurrence could play a valuable role in guiding day-to-day management decisions. Collation of information on available models and research results would provide useful guidance for Member States.

3.4 Wider/emerging issues

Recommendations

1. At locations where swimmer's itch is known to occur, this should be included in the bathing water profile and information provided to members of the public.
2. Where cases of wound infection (e.g. caused by *Vibrio* spp.) have resulted from a recreational water exposure, this information should be communicated in the bathing water profile. In addition, on-site information should be provided including advice on bather hygiene measures to minimise risk and actions to take if a wound is sustained while bathing.

Good practice

3. Surveillance of surface waters for antimicrobial resistance is in the development phase and is, therefore, not yet easy to make obligatory in a regulatory context. Source control and treatment options may be a useful strategy in the interim and liaison with the European Medicines Agency and the authorities implementing the Urban Waste Water Treatment Directive is suggested.
4. The issue of microplastics falls within the scope of the Marine Strategy Framework Directive and microplastics should be considered under that Directive until additional information becomes available to assess the possible importance to the BWD.

5. It is suggested that the potential application of the BWD (which is currently restricted to bathers) to other recreational activities is considered if the non-bathing use of sites continues to increase.

3.4.1 Swimmer's itch

Swimmer's itch (also known as cercarial dermatitis) is an unpleasant skin reaction caused by exposure to a freshwater parasite (schistosome). Cases have been reported in a number of European countries (see E3.1) and it may be locally common. Avian schistosomes have a complex lifecycle involving freshwater snails and waterfowl, which means that eradication of the problem is difficult. Where cases have been identified, this should be included in the bathing water profile and information provided to members of the public.

3.4.2 Wound infection

A number of microorganisms can cause wound infections following exposure to bathing waters (see Sections E3.2 and E3.3). Where cases of wound infection have resulted from bathing water exposure the information should be recorded in the bathing water profile and on-site advice given on bather hygiene measures to minimise risk (such as covering an existing wound with a waterproof plaster prior to immersion) and actions to take if a wound is sustained while bathing.

3.4.3 Antimicrobial resistance

Bathing water is not thought to be a major route of transmission for antimicrobial resistant microorganisms and environmental surveillance techniques are not currently sufficiently advanced for obligatory routine monitoring (see E1). It is suggested that the most appropriate measures to take relate to source control and treatment options and therefore, liaison with the European Medicines Agency and the authorities implementing the Urban Waste Water Treatment Directive is advised.

3.4.4 Microplastics

Microplastics are a relatively new area of concern (see E2) and there are, currently, a number of unanswered research questions in relation to whether they are likely to be relevant to the BWD. Irrespective of the state of research they already fall within the scope of the Marine Strategy Framework Directive.

3.4.5 Other recreational water users

The BWD is currently restricted to 'bathers'. Extension to include other recreational water users (e.g. sailors, surfers and so on) would mean a requirement for additional monitoring sites, an extension to the bathing season and, possibly, the zoning of designated sites (see E4). It is suggested that widening the scope of the BWD is reconsidered in the future if the non-bathing use of bathing sites continues to increase.

4. Fact sheets

The following fact sheets are intended as stand-alone sections. There is, thus, some repetition between some of the sections (most notably for the current parameters).

A. Current parameter – intestinal enterococci

A1. Introduction

Intestinal enterococci (ENT) are Gram-positive spherical or ovoid bacteria arranged in pairs or chains, and are members of the genus *Enterococcus*. They were previously classified in the genus *Streptococcus* and some of the earlier literature refers to them as faecal streptococci. For the purposes of environmental monitoring, faecal streptococci and ENT are considered to be largely synonymous [(1)].

ENT are commensal bacteria and they are shed in high numbers in human and animal faeces (e.g. 10^2 to 10^8 bacteria/ gram of dry faeces [(2)]). As a result, they are easily detected in contaminated water and their use as a faecal indicator organism (FIO), where their presence in water indicates possible faecal contamination, is long-standing.

Despite their widespread use, ENT have some potential drawbacks in water quality monitoring. For example, they have a number of environmental habitats that can serve as both sources and sinks of ENT. Particularly relevant to beaches are some ENT members that may be endogenous in sediments and soils and not exclusively of faecal origin [(1)].

A2. Current situation

ENT is the only parameter suggested by the WHO guidelines [(3)] and is currently used as a regulatory parameter in both the European Union (EU) Bathing Water Directive (BWD) and a number of other recreational water regulations throughout the world, outlined in Table A1 (although many of these regulations are currently under review). The BWD is the only set of major regulations that requires the measurement of both *Escherichia coli* (*E. coli*) and ENT at monitored sites. The BWD classification is based on percentile measurements, with the calculation method (which assumes that the data are $\log_{10}$ normally distributed) given in Annex II.

Results of a Member State questionnaire survey and discussions from an EC stakeholder consultation meeting with the technical input from the WHO suggested that the use of both a 95-percentile (excellent, good) and 90-percentile values (sufficient) in the BWD classification is seen as confusing and difficult to justify. There was clear support for the use of 95-percentile values to be used across all the classifications.

Table A1: WHO guidelines and selected regulatory levels for ENT in recreational water

Water type	Acceptable water quality/100ml (measure)	Comments	Status	Organization
Fresh and marine	≤ 500 cfu with low to moderate susceptibility to faecal influence (95th percentile)	Based on the lower value for a rating of 'fair' (estimation of up to a 10% GI illness risk)	G	WHO [3]
Fresh and marine	≤35 cfu (GM) and ≤130 cfu (90th percentile) ≤70 cfu (75th percentile) ***or using qPCR*** 470 CCE (median) or 2000 CCE (90th percentile) 1000 CCE (75th percentile)	Based on a GI illness rate of 36/1000 Optional beach action value (BAV) Choice of ENT **or** *E. coli* for fresh water	R	USEPA [4]
Fresh	≤330 cfu (90-percentile) ≤400 cfu (95-percentile) <200 cfu (95-percentile)	Based on 'sufficient' classification Based on 'good' classification Based on 'excellent' classification Measurements for *E. coli* also required	R	EU [5]
Marine	≤185 cfu (90-percentile) ≤200 cfu (95-percentile) <100 cfu (95-percentile)	Based on 'sufficient' classification Based on 'good' classification Based on 'excellent' classification Measurements for *E. coli* also required	R	EU [5]
Marine	≤35 (GM) ≤70 (single sample max)	Minimum of 5 samples	R	Health Canada [6]

G: guideline R: regulation GM: geometric mean cfu: colony forming units qPCR: quantitative polymerase chain reaction CCE: calibrator cell equivalents GI: gastrointestinal USEPA: United States Environmental Protection Agency

A3. Epidemiological data

Epidemiological studies are used to evaluate illness resulting from exposure to contaminants and/or activities and have been used to inform recreational water quality guidelines and regulations. The studies typically evaluate the levels of illness in swimmers (or other water recreators) and non-swimmers and relate the illness rates to the exposure (usually characterised by levels of FIO). Results are typically expressed as odds ratios (OR) or other types of relative risks (RR) and there is a statistically significant increase in risk between the groups if the lower 95% confidence interval (95% CI) is greater than one (approximately corresponding to a p-value of <0.05). Studies usually examine a range of possible illnesses, such as gastrointestinal (GI) illness, respiratory problems, eye, ear and skin symptoms. The exact definitions of the illnesses and symptoms vary between studies.

While epidemiology relating to swimming exposure dates back to the 1940s (USA) and 1950s (Europe), this fact sheet focuses on studies which were used to inform the WHO Guidelines [3,7], large European studies and work published since 2009.

The microbial water quality criteria for the WHO Guidelines [3] were derived from a series of epidemiological studies conducted with adults in UK sewage-contaminated coastal waters [8-9]. These studies were designed to avoid potential biases resulting from the design of earlier studies by using a randomized-trial design. Participants were recruited in advance of the trial and then randomly

allocated, on the study day, to either a bathing or non-bathing group (to avoid self-selection bias), each bather was asked to spend at least ten minutes in the water and immerse their heads three times. Extensive water quality monitoring was conducted during the trial and microbial water quality closest to the time and place of exposure ascribed to individual bathers, thus giving an accurate assessment of exposure. Only ENT (measured as faecal streptococci) measured at chest depth showed a statistically significant dose-response relationship for any illness. Dose-response relationships were seen for GI illness (faecal streptococci levels above 32/100ml) and acute febrile respiratory illness (AFRI – faecal streptococci levels above 60/100ml). The variability in FIO was taken into account when calculating the burden of disease attributable to recreational water exposure by combining the dose-response relationship with a probability density function describing the distribution of FIO. This allowed for both the mean and variance of the bacterial distribution to be taken into account.

In Europe, a randomised control trial was conducted at five freshwater sites in Germany (four lakes and one riverine site); sources of faecal contamination included treated and untreated municipal sewage, agricultural runoff and water fowl [10]. Relationships were demonstrated for three different definitions of GI illness and ENT and *E. coli*. Relative risk values depended on the definition of GI illness and ranged from 1.8 (95% CI 1.2-2.6) to 4.6 (95% CI 2.1-10.1).

Epibathe was a European-based study which was specifically designed to address the "*relative paucity of EU data describing the health effects of bathing in EU freshwaters and Mediterranean marine waters*" [11]. Eight separate randomised control trials were completed, four at different freshwater sites in Hungary and four at two different marine beaches in Spain. The results from these trials were analysed both separately and in combination with the existing data acquired using the same methodology [8, 10]. The risk of GI illness was higher in bathers (compared to non-bathers) in both the Spanish and Hungarian studies, although not significantly so. Analysis of the combined data set (using meta-analysis), specifically the GI symptoms, suggested that ENT was the best predictor of illness in bathers using marine waters (combined data OR 1.38; 1.03-1.87) and that *E. coli* may be a better index of GI symptoms in bathers using freshwater (combined data OR 1.19; 0.88-1.62). Results from the freshwater studies, however, were not statistically significant and did not show a consistent exposure-response association (i.e. an incremental increase in illness with increasing FIO exposures).

The evidence from the European studies and other international research outputs (both published and in progress) was considered at an international expert meeting in 2009. The resulting output was an addendum to the 2003 Guidelines for safe recreational water environments [7], which concluded that no change was required to the current WHO water quality Guidelines [3].

The studies published from temperate locations since the update to the WHO Guidelines [7] are summarised for GI illness (the most commonly reported outcome) in Table A2 [12-23]. It can be seen from this Table that much of the recent epidemiological research has focussed on beaches affected by non-point source pollution and that, typically, the studies have only shown a dose-response relationship between health outcome and ENT levels when there was significant human input [14, 16].

Table A2: Summary of epidemiological studies (2009-2017) conducted in temperate locations and relationships with ENT

		Beaches (n)					
Country (Reference)	**Study type**	**Pt source**	**Non-pt source**	**Summary water quality (ENT)**	**Overall GI effect***	**Relationship between GI & ENT**	**Comments**
Marine water							
USA [12]	PC	3	-	GM (max) cfu/100ml Edgewater 7 (920) Fairhope 21 (3,000) Goddard 4 (960)	√	√ Daily ave ENT (by PCR) & GI: AOR: 2.6 (1.3-5.1)	
USA [13]	Ran	-	1	Mean (max) cfu/100ml 71 (3,320)	x	x	
USA [14]	PC	1 (Int.)		Median cfu/100ml (close to creek input): Berm open 316 Berm closed 10	√/x	√/x Daily ave ENT (culture & PCR) & GI: AOR 2.5 (1.5-4.1) cultured ENT (berm open)	Effects seen when the berm was open (point source discharge)
USA [15]	PC	-	1	GM (max) cfu/100ml: 3 (1,740)	√	x	
USA [16]	PC	1 (Int.)		GM (max) cfu/100ml: 30 (>10,000)	√/x	√/x ENT (culture) & GI: AOR 1.85 (1.1-3.2) swallowed water, SGD operating	Relationship seen when the SGD operating
USA [17]	LC		2	No summary measures given, ENT was significantly higher at 5 of the 6 sample points post rainfall	x	x	Beaches affected by urban runoff, winter study in surfers
Greece [18-19]	PC		3	GM (max) cfu/100ml Beach A: 6 (1,380) Beach B: 3 (74) Beach C: 3 (15)	√	x	Symptoms thought to be related to bather density
Denmark [20]	RC			FIO peak cfu/100ml based on modelled data 2010: ENT 6,000 2011: ENT <200	√	x	GI effect seen in 2010 vs 2011 participants and in 2010 water swallowers vs non-swallowers
Fresh water							
USA [21, 22]	PC	CAWS	GUW	Mean cfu/100ml CAWS: 200 GUW: 71	√	x	Limited-contact water recreation
Netherlands [23]	PC	2		Utrecht (U) – no data Amsterdam (Am) max cfu/100ml ENT: 100	U x Am √	Not determined GI & self-reported water swallowed	Amsterdam site subject to sewer flooding 2 days before the event

* Overall GI effect seen between bathers versus non-bathers PC: prospective cohort Ran: randomised control trial LC: longitudinal cohort RC: retrospective cohort GM: geometric mean Int. intermittent SGD: submarine groundwater discharge Predom: predominantly CAWS: Chicago area waterways system GUW: general use waters

In addition to the studies outlined in Table A2, a number of combined analyses have also been performed. Skin symptoms in swimmers versus non-swimmers at FIO levels above and below the USEPA [4] recommended threshold levels were compared [24]. Twenty studies were analysed (nine freshwater, eleven marine) and statistically significant results were reported for ENT and *E. coli* for marine sites.

An analysis of 13 prospective cohort studies (conducted at both fresh and marine sites in the USA), with a combined number of participants of over 84,000, has recently been published [25]. The incidence of diarrhoea was found to be higher in individuals with body or head immersion compared to non-swimmers. The incidence increased further in those people who reported swallowing water. Swimming exposure above the USEPA regulatory guideline (ENT >35 cfu/100ml) increased diarrhoea incidence only at beaches with a known point source of human faecal contamination.

A pooled analysis of six prospective cohort studies (including four of the studies [12, 14-16] outlined in Table A2) set at marine beaches in the USA, examining the relationships between GI illness, ENT and coliphages was recently reported [26]. The exposure days were classified according to whether human faecal contamination was likely to be present. Under all conditions (i.e. not accounting for presence of contamination) there was no association between GI illness and swimming in water containing detectable coliphages and ENT. When human faecal pollution was present, however, coliphage and ENT were associated with increased GI illness and there was some evidence that F-specific phage had a stronger association with illness than ENT under those circumstances.

A4. Water quality analysis

Methods for the analysis of bathing water quality have, traditionally, been based on culture techniques, where the target bacteria in the water sample are grown using selective media and suitable incubation temperatures. Distinctive features, such as growth at 44 °C and expression of specific enzymes, are used for positive identification and results are presented as the number of target bacteria per volume of water (usually 100 ml). As bacterial growth is required, culture techniques typically require at least 18 hours before the results are available and so there has been a move to develop alternative methods which can provide more rapid results.

The most commonly used molecular method is quantitative polymerase chain reaction (qPCR), which works through the *in vitro* amplification of specific segments of the genome (DNA or RNA) from the microorganism in question. To date, there are two related recreational water regulatory approved qPCR methods (ENT: Method 1611 [27] and Method 1609 [28]) and the use of qPCR was supported by the results of epidemiological studies conducted in the USA (at sewage-impacted beaches) which showed the strongest relationship between bather health and ENT measured using Method 1611 [12, 29].

Key requirements for analytical methods are sensitivity (the ability to detect small numbers of the target organism) and specificity (the ability to detect only the target organism) and, in addition, methods need to be repeatable (within a laboratory) and reproducible (between laboratories). It is also useful to consider the complexity of the test (which will have implications for staff training), the need for specialised equipment, the cost-benefit analysis and the time required to get accurate results [30].

The BWD stipulated a choice of two International Organization for Standardization (ISO) methods, based on culture techniques, for ENT (ISO 7899-1 [31] and 7899-2 [32]). Member States can, however, use alternative methods providing that the alternative method's equivalence to the reference method is demonstrated.

The methods [31, 32] aim to isolate and enumerate the major intestinal ENT; other ENT species may also occasionally be detected, although their presence is expected to be low. The Part 1 method [30] is considered to be applicable to all types of surface and waste waters, particularly those containing significant particulate material. It is not suitable for use where the expected ENT concentration is less than 15 per 100ml. The Part 2 method [32] is best suited to drinking-water, water from swimming pools or other disinfected/clean water sources, although it can be applied to all types of water (except where they contain high levels of suspended solid or high levels of interfering bacteria).

The USEPA have developed methods [27, 28] for the enumeration of ENT in recreational waters using qPCR (Method 1611 and its modification, Method 1609). In Method 1609 (and Method 1611 stipulated in the 2012 USEPA regulations [4]), ENT target-DNA sequences, present in the sample (based on a specific region of the 23S ribosomal RNA), are detected by qPCR using TaqMan® 'environmental master mix' (Method 1611 uses 'universal master mix') PCR reagent and probe system. This system signals the formation of PCR products by a process involving enzymatic hydrolysis of a fluorogenically-labelled oligonucleotide probe when it hybridizes to the target sequence [27]. Results are expressed as calibrator cell equivalents (CCE) per 100 ml. Method 1609 includes an internal amplification control. The method notes that during validation studies, highly variable recoveries were seen, which should be taken into account when considering the results. It is suggested that site-specific analysis of the method's performance should be conducted before it is used for beach notification, or advisory programmes [4]. In a comparison of methods [33] using river water samples, Method 1609 was found to be more resistant to inhibition than Method 1611, although the authors concluded that both methods should be suitable for comparison with the USEPA [4] values for qPCR measured ENT. Presently, USEPA recommends Method 1609 over Method 1611 because sample dilution is generally not required in order to reduce interference issues and it has a lower overall interference rate. In Canada, improved ENT detection sensitivity and reduced interference has been demonstrated with Method 1609 when using a 20 ml (rather than 100 ml) sample.

A5. Bathing water profile

Bathing waters designated under the BWD require a bathing water profile (Annex III). This includes identification and assessment of pollution (and its causes) that could impact on both water quality and bather health. The profile is, principally, intended to lead to an understanding of the faecal sources and pollution routes impacting a site. This can be used to plan appropriate management measures and as a source of information to communicate bathing water quality information. There are a number of tools which may assist in aspects of conducting a bathing water profile, including detailed water quality studies, faecal source attribution (including microbial source tracking - MST) and quantitative microbial risk assessment (QMRA).

A5.1 MST

The idea behind MST is that genetic markers within certain faecal microbes are strongly associated with specific hosts (e.g. humans, livestock, dogs and gulls) and that certain identified attributes of

those microbes can be used as markers for faecal contamination from that host [34]. Table A3 lists some commonly used MST targets and their associated hosts that have been used for investigation of recreational water.

Table A3: MST targets and associated hosts

Human	Cow/ruminant/pig	Gull	Dog
Human viruses:	CowM2	Gull2	DogBac
Enterovirus - EV	CowM3	LeeSeaGull	BacCan
Adenovirus - AdV	BacCow	Gull4	
Norovirus (GI) - NovGI	BacR		
Norovirus (GII) - NoVGII	Rum2Bac		
Polyomavirus JC – PyV-JC	Bovine AdV - BAdV		
Polyomavirus BK – PyV-BK	Bovine PyV - BPyV		
HF183	Pig2Bac		
BacHum	Porcine AdV - PAdV		
HumM2			
Lachno2			
HB			

MST has been applied to a number of bathing waters and the techniques have been successfully used to guide beach management / remediation decisions, where targeted interventions have led to a reduction in beach FIO concentration [35-37].

As the presence of human faecal contamination seems to be necessary for an ENT dose-response relationship (see A2), the USEPA have developed MST markers, measured using qPCR, to facilitate the identification of human sewage [38]. Where sanitary survey and the use of MST markers shows that recreational waters are free from sewage and other faecal matter of concern (e.g. from ruminants), the USEPA allows for the setting of a site specific FIO target level [4].

While the concept behind source tracking is conceptually clear, the application of techniques and interpretation of results is work in progress [39]. Ideally, source apportionment using MST would allow just that, the knowledge that (say) 15% of FIO are derived from human sources, 75% from gulls and up to 10% from dogs and other unspecified sources. Unfortunately, such quantification currently relies on a number of assumptions [39-41], which often are not fully met or are untested, including:

- host-specific markers are host-specific and do not cross react with other species;
- host-specific markers have similar environmental survival rates, fate and transport;
- the species of interest shed a similar amount of its host-specific markers;
- the FIO: marker relationship is similar between species and markers;
- each host-specific marker has a similar prevalence and proportional distribution among individuals within the species.

A5.2 QMRA

QMRA consists of four steps (hazard identification, exposure assessment, dose-response assessment and risk characterization), with data for each of the steps drawn from an appropriate mix of the published literature, site-specific measurements and clearly documented assumptions [42]. The application of QMRA to recreational water can be used to investigate a range of different scenarios and management questions (in a hypothetical manner), and can be used to augment and complement epidemiological studies [43] and to improve routine bathing water monitoring and management [44]. Some of the questions posed by recent recreational water QMRAs include [43, 45-53]:

- What sources of faecal contamination are likely to represent the greatest risk of infection?
- What is the impact of mixed faecal contamination on illness risk and allowable levels of ENT?
- What pathogens are likely to cause the illness rates seen in an epidemiological study?
- What is the health impact of incidental contact recreation from freshwater receiving secondary treated (but non-disinfected) effluent?
- What is the risk of illness from specific pathogens possibly present in bathing waters (e.g. *Cryptosporidium*, *Giardia* and adenovirus)?
- What is the impact of storm water/wet weather on the risk of recreational water related GI illness?
- What concentrations of MST markers suggest a bather GI illness rate of (for example) 30/1000 swimmers?

The results of these studies highlight the importance of viruses as a key cause of recreational illness, and they provide support for the greater risk posed by human (and also bovine) faecal contamination, and indicate the importance of rainfall in increasing incidence of illness and suggest possible reasons why epidemiological studies may not always find a relationship between FIO and swimmer health.

The use of QMRA within a regulatory framework is currently being trialled in California (USA) and Alberta (Canada) where, in the absence of human or bovine MST markers, QMRA may be used to develop site-specific faecal indicator levels using the approaches described by Schoen *et al*. [45].

A6. Prediction (for daily beach management decisions) and discounting

Where recreational water is subject to occasional and predictable deterioration (such as after rainfall) and where users can effectively be discouraged from entering the water during such periods (e.g. through signage/beach advisory notices), the WHO Guidelines [3] suggest that the classification may be upgraded to reflect the water quality that users are actually exposed to during periods not covered by 'advisory' signage, providing that there is accompanying explanatory material. Thus, results from water quality samples taken during this period can be discounted from the overall classification.

Modelling has been put forward as a means of facilitating the prediction of periods of poor water quality, enabling timely (near-real-time) and appropriate information to protect public health [7]. A number of model types have been investigated for use in recreational water quality prediction [e.g. 54-61] including Multiple Linear Regression (MLR), Artificial Neural Networks (ANN), decision tree and hydrodynamic modelling, with MLR being the most commonly applied for daily beach management decisions. To be useful management tools, predictive MLR models should achieve an explained variance (R^2 value) of >60% with well documented control of multicollinearity [62]. Where this could not be achieved through simple black box modelling then further investigation of the contributing catchments and their human and animal microbial flux through budget studies, often termed quantitative microbial source apportionment - QMSA [63], was recommended [62]; possibly with the parallel application of more complex and process-based hydrodynamic modelling better to determine the linkage from the multitude of input fluxes to the impacted bathing water sites [64, 65].

Statistical models use observed 'associations' between impaired water quality and measurable environmental parameters in the antecedent period leading up to the prediction. Observed associations do not prove 'causation' between the environmental variable and the change in water quality. Causation and the implied physical connectivity can be investigated further through tracer

studies using microbial (e.g. phages) and/or dye (e.g. Rhodamine WT) tracers. These are generally used in conjunction with QMSA investigations to define flux from a multitude of FIO sources potentially impacting upon a bathing water location [66, 67]. Simple rainfall thresholds were investigated in the development of early UK prediction of bathing water quality [68] and, in some cases, can be effective. However, it is generally true that the drivers of FIO concentration in recreational waters are more complex than can be characterised by a single predictor. It is for this reason that the most common statistical model applied to bathing water prediction is an MLR model. These are commonly available in commercial software systems which allow for parametricity testing of the raw data to ensure the data are appropriate for the statistical approach employed.

Most of the black box statistical modelling systems in use today (e.g. the US Virtual Beach and Nowcast software, the UK and Portugal [68, 69]) predict the water quality on the bathing day through one, early morning, model run, on which any public advisories (warnings) are based.

The principal strength of the MLR approach is that it can be built using regulatory (FIO) data and archive data describing candidate predictor variables. Thus, it can be applied without the requirement for new microbial data acquisition in most cases. Its main weakness is the implicit assumption that water quality on the bathing day is characterised by a single sample and is constant. This assumption has been questioned [70, 71]. Indeed, recent investigation at two UK sites subject to intensive sampling (half hourly samples throughout the bathing day for 60 days during the bathing season) observed ten to 1000 fold variations in FIO, with significant diurnality at one site surveyed.

Although modelling costs (especially where data acquisition for dependent and predictor variables is required) are perceived to be high, model implementation has the potential to enhance the chance of a beach complying with water quality standards (through discounting), reduce the impacts on availability/use of the beach (with the associated impacts on tourism and local beach-side economies) and potentially provide significant cost savings as managers are not forced to seek to reduce FIO loading during peak events to see a rapid improvement in both public health protection and compliance.

A7. Classification

The current EU bathing water classification requires an assessment of both ENT and *E. coli*, as shown in Table A4, and is based on results from a four-year period (or three-year period provided the conditions set in the BWD are met) and should consist of at least 16 samples (although an assessment of a newly identified bathing water can be based on results from a shorter period providing that the requirements for the minimum number of samples has been met). Samples are taken, immediately before and then, at least monthly, throughout the bathing season.

Samples taken during short-term microbiological pollution (affecting the bathing water for normally no more than 72 hours) can be discounted as long as a number of requirements are met, these include ensuring that bathers are deterred from entering the water during that period, an additional sample is taken after the end of the pollution of the affected bathing water to replace the disregarded sample and a stipulation that no more than one sample per year or no more than 15% of samples from the assessment period (whichever is greater) fall into this category.

Table A4: EU Bathing Water Directive standards for recreational water and classification results for 2015 & 2016 [5, 72, 73]

Parameter	Excellent quality	Good quality	Sufficient	Poor	No classification^
Inland waters					
ENT (cfu/100ml)	200 (*)	400 (*)	330 (**)		
E. coli (cfu/100ml)	500 (*)	1000 (*)	900 (**)		
Coastal & transitional waters					
ENT (cfu/100ml)	100 (*)	200 (*)	185 (**)		
E. coli (cfu/100ml)	250 (*)	500 (*)	500 (**)		
Bathing water classification					
2016 classification (%)	85.5	8.4	2.4	1.4	2.3
2015 classification (%)	84.4	9.1	2.6	1.6	2.3

(*) based upon a 95-percentile evaluation (**) based upon a 90-percentile evaluation ^ quality classification not possible

It can be seen from Table A4 that the majority of EU bathing waters are classed as having excellent or good water quality, with less than 3% being 'sufficient' and less than 2% 'poor'. The percentage of both fresh and marine bathing waters achieving excellent quality (or complying with the guide values from the earlier Directive) has steadily been increasing although, overall, inland sites lag behind marine sites.

Preliminary results from a questionnaire survey of Member States suggests that, overall, the classification of marine waters is more likely to be driven by concentrations of ENT than by *E. coli*. A check of the data available for the 2016 bathing season assessment, however, indicated that for most of the marine sites both parameters were important, whereas for the fresh water classification the principal driver is *E. coli*.

Results from the 2016 bathing season monitoring show that 516 bathing waters (336 coastal and 180 inland) are classed as 'sufficient'. A questionnaire survey of Member States suggested that if the sufficient classification was removed it was likely that a number of these beaches would be de-designated. This is likely to have not only immediate negative effects on the relevant local communities, but also longer term negative consequences such as a further reduction in water quality following the removal of active beach management [74].

A8. Conclusions

The stated purpose of the BWD is "*to preserve, protect and improve the quality of the environment and to protect human health*" (Article 1). The focus in this fact sheet and the WHO recommendations, however, is solely on health protection.

ENT should be retained within the BWD. There is sound epidemiological data supporting its use, as shown in Section A3 and Table A2.

The four levels within the classification system (excellent, good, sufficient and poor) should be retained. The 'sufficient' category provides impetus for progressive improvement and it is possible that, if this category were removed, a significant number of the beaches may be de-designated (see Section A7).

The classification system currently uses different assessment methods. 'Excellent' and 'Good' are based on a 95%ile value, while 'sufficient' is based on a 90%ile. This is confusing and difficult to explain to the public. It is recommended that all of the categories should be based on a 95%ile value;

this retains consistency (for the most part) with the existing BWD and is in line with the WHO Guidelines approach. Based on a standard deviation of 0.8103 (derived from data from 11000 EU bathing waters and the value from which the WHO guidelines for marine water were calculated [75]), the ***equivalent*** values for 'sufficient' are shown in Table A5. These values retain the same level of health protection as the current sufficient classification.

Table A5: Recommended changes to BWD sufficient classification for Enterococci

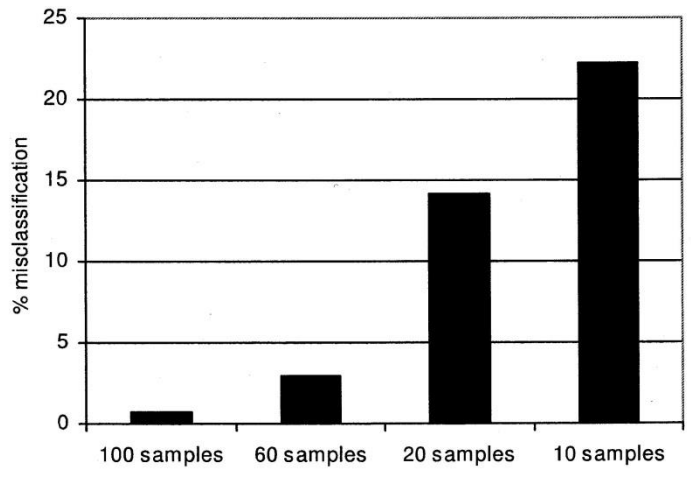
	Sufficient (cfu/100ml)	
Water type	**Current (90%ile)**	**Amended (95%ile)**
Marine	≤185	≤367
Fresh	≤330	≤656

Amended values based on a standard deviation 0.8103

The current minimum sample number for overall classification (16) leads to significant (i.e. 15-20%) misclassification of bathing water locations, as shown in Figure A1 [7]. This is considered unacceptable for a standard designed both:

(i) to communicate public health information to the general public; and
(ii) to define the legal compliance of bathing water to EU regulators and Member States.

In public health terms, using the epidemiological approach in the WHO Guidelines for safe recreational water environments (2003), misclassification of a 'Poor' bathing water as 'Good' would imply an actual health risk of gastroenteritis exceeding 8.4% when the bathers should be assured of a health risk of 3-5% which could reduce confidence in the regulatory agencies concerned.



Based on hypothetical data, using parametric 95th percentile values and assuming a standard deviation of log_{10} values of 0.8

Figure A1: Misclassification rates in bathing waters [7]

To avoid both the adverse health effects experienced by bathers of a poor bathing water misclassified as compliant, and the economic costs to local community businesses of a compliant bathing water being misclassified as poor, the ***annual*** minimum sample number collected in the bathing season in EU bathing waters should, therefore, be increased to 20 samples per site, with the overall classification being based on at least 80 samples collected over four years. In some cases

(where locations have not undergone any major changes which are likely to change microbial levels), it may be appropriate to base the classification on more than four-years of data (in order to reach the new minimum sample numbers). It is noted that time will need to be allowed for the countries taking the current annual minimum number of samples (four) to adjust to the new sampling regime (20). Where significant infrastructure investments are made at an existing site (hopefully producing a 'step-change' improvement in water quality), it is expected that only sample data produced since the improvement (as required in the BWD) will be used: this will involve an unavoidable reduced sample number for interim compliance assessment.

Recreational water quality data is not always log_{10} normally distributed [76, 77] (although this tends to be the assumed position). The data from bathing water sites with at least 80 samples should be tested for log_{10} normality, using the Shapiro-Wilk test. Where log_{10} normality is demonstrated, the calculation method used in Annex II of the BWD can be used. Where data is not shown to be log_{10} normally distributed, the Hazen method of calculation should be used to calculate 95%ile values [7]. Log_{10} normality should be reviewed annually using the full data sequence to be used for compliance assessment. For sites without the required number of samples, it is suggested that it is assumed that the data is ***not*** log_{10} normally distributed (and thus the Hazen method should be used until enough samples have been analysed).

Accreditation determines the technical competence and integrity of organisations offering a range of services, including microbiological analysis. Sampling and sample analysis should be conducted by laboratories which are accredited for the methods being used. Detection levels can impact on the beach classification. Member States currently have to use the ISO methods specified in the BWD (or methods with demonstrated equivalency), the limits of determination (LoD) should be based on the specified test method (e.g. 3/100ml for membrane filtration and for some of the most probable number methods and 15/100ml for the microplate MPN method). Advice is available on appropriate dilution practices [e.g.78] and accredited laboratories should be able to consistently achieve these levels.

A wide range of molecular methods have been developed (such as qPCR), however, it is not currently recommended that these be used for regulatory purposes in the BWD as dose-response data using these methods have not been obtained from European study sites and the methodology is not yet mainstream. If European-based epidemiological studies are conducted in the future, it is suggested that qPCR methods for ENT and *E. coli* (and possibly enteric virus and MST markers) are included in the water quality microbial analysis suite to examine their possible suitability for European regulation.

Many recreational waters exhibit marked spatial and temporal variability. The BWD currently recommends that the monitoring point should be the location where most bathers are expected, or where the greatest risk of pollution is expected (according to the bathing water profile). In terms of spatial variability across the designated protected bathing area, it is suggested that the water quality should be representative of the whole bathing area (demonstrated within the bathing water profile); this could be confirmed by annual, spatial/beach shoreline transect sampling. Temporal variability could be addressed by sampling at different times of the day, or taking a precautionary approach and sampling in the morning, when water quality is generally poorer [79], unless it is influenced by bather contamination.

Predictive modelling (Section A6) can provide information on daily beach management decisions (e.g. notification of the public that the water is not suitable for bathing). While their use is not currently widespread their potential contribution to public health is noted. Where they are employed, the model type and predictive variables used (e.g. rainfall) should be the choice of the Member States. The choices of models and methods of public information dissemination should be reported to the EC and the models employed should meet minimal requirements (including an explained variance of ***at least*** 50-60%) and the approach taken should be justifiable and auditable. The models should be optimized to predict the higher end microbial concentrations. The results should be used to inform the public, rather than for regulatory purposes, although where public 'informed-choice', at the time of predicted high results, can be demonstrated (e.g. through timely signage and electronic communication tools), they could also allow the discounting of water sample results taken at the time of the event (within current BWD specified allowances). Research is needed on the minimum number of data points required for model building and also whether prediction models could be used instead of a check sample to allow a return to use after a short-term pollution event.

Threshold values may provide guidance on site re-opening (i.e. de-warning), but they are likely to be site specific and, thus, it is felt that they are not amenable to regulatory use.

While methods such as MST and QMRA provide useful information for the bathing water profile, their use should be optional. It would be valuable to commission a detailed state of the art review, to provide standardised information and advice on their practical application to Member States.

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B. Current parameter - *Escherichia coli*

B1. Introduction

Escherichia coli (*E. coli*) is a Gram-negative, oxidase-negative, rod-shaped bacterium of the family *Enterobacteriaceae*. It is part of the group of bacteria known as thermotolerant coliforms. *E. coli* is abundant in human and animal faeces (comprising approximately 1% of the total bacterial biomass [(1)]) and is generally present in greater numbers than enterococci (ENT) in fresh excreta.

E. coli has been described as an innocuous resident of the gastrointestinal tract although, while for the most part that is true, there are also some strains that are pathogenic and which can cause significant diarrhoeal and extra-intestinal illness [(2)], yet such strains are not targeted by most routine water quality culture media.

Despite a long history of use as a faecal indicator organism (FIO), *E. coli* was first introduced as an indicator of faecal contamination in 1893 [(1)], it has been isolated from tropical water systems and effluents from pulp and paper mills with no known sources of faecal contamination [(1)] and research has shown that naturalised *E. coli* populations do exist [(3)]. As these naturalised *E. coli* can be present in soil, sand and sediment of coastal watershed they may confound the relative risk estimate of these FIO as currently used in beach monitoring programmes.

B2. Current situation

E. coli is currently used as a regulatory parameter in both the European Union (EU) Bathing Water Directive (BWD) and a number of other recreational water regulations throughout the world, as summarised in Table B1 (although many of these regulations are currently under review). The EU BWD is the only set of major regulations that requires the measurement of both *E. coli* and ENT at all monitored sites. The BWD classification is based on percentile measurements, with the calculation method (which assumes that the data are $\log_{10}$ normally distributed) given in Annex II.

Although the measurement of *E. coli* in marine waters and the requirement for both *E. coli* and ENT to be measured at all sites was queried at a European Commission (EC) stakeholder meeting its use was, overall, felt to provide useful information. It drives compliance with the BWD at some sites, it provides information on recent contamination, its continued measurement supports temporal trend analyses and it may, potentially, play a role in future issues (such as investigations into antimicrobial resistance).

Table B1: Selected regulatory levels for *E. coli* in recreational water

Water type	Acceptable water quality/100ml (measure)	Comments	Status	Organization
Fresh	≤126 cfu (GM) and ≤410 cfu (90th percentile) ≤235 cfu (75th percentile)	Based on an illness rate of 36/1000 Optional beach action value (BAV) Choice of ENT **or** *E. coli* for fresh water	R	USEPA [4]
Fresh	≤200 cfu (GM) ≤400 cfu (single sample max)	Minimum of 5 samples	R	Health Canada [5]
Fresh	≤900 cfu (90-percentile) ≤1000 cfu (95-percentile) <500 cfu (95-percentile)	Based on 'sufficient' classification Based on 'good' classification Based on 'excellent' classification Measurements for ENT also required	R	EU [6]
Marine	≤500 cfu (90-percentile) ≤500 cfu (95-percentile) < 250 cfu (95-percentile)	Based on 'sufficient' classification Based on 'good' classification Based on 'excellent' classification Measurements for ENT also required	R	EU [6]

R: regulation GM: geometric mean USEPA: United States Environmental Protection Agency cfu: colony forming units ENT: enterococc

Results of a Member State questionnaire survey and discussions from an EC stakeholder meeting suggested that the use of both a 95-percentile (excellent, good) and 90-percentile values (sufficient) in the BWD classification is seen as confusing and difficult to justify. There was clear support for the use of 95-percentile values to be used across all the classifications.

B3. Epidemiological data

Epidemiological studies are used to evaluate illness resulting from exposure to contaminants and/or activities and have been used to inform recreational water quality guidelines and regulations. The studies typically evaluate the levels of illness in swimmers (or other water recreators) and non-swimmers and relate the illness rates to the exposure (usually characterised by levels of FIO). Results are typically expressed as odds ratios (OR) or other types of relative risks (RR) and there is a statistically significant increase in risk between the groups if the lower 95% confidence interval (95% CI) is greater than one (approximately corresponding to a p-value of <0.05). Studies usually examine a range of possible illnesses, such as gastrointestinal (GI) illness, respiratory problems, eye, ear and skin symptoms. The exact definitions of the illnesses and symptoms vary between studies.

While epidemiology relating to swimming exposure dates back to the 1940s (USA) and 1950s (Europe), this literature review focuses on that which was used to inform the WHO Guidelines [7, 8], large European studies and work published since 2009.

The microbial water quality figures for the WHO Guidelines [7] were derived from a series of epidemiological studies conducted with adults in UK sewage-contaminated coastal waters [9, 10]. These studies were designed to avoid potential biases resulting from the design of earlier studies by using a randomized-trial design. Participants were recruited in advance of the trial and then randomly allocated, on the study day, to either a bathing or non-bathing group (to avoid self-

selection bias), each bather was asked to spend at least ten minutes in the water and immerse their heads three times. Extensive water quality monitoring was conducted during the trial and microbial water quality closest to the time and place of exposure ascribed to individual bathers, thus giving an accurate assessment of exposure. Only ENT (measured as faecal streptococci) measured at chest depth showed a statistically significant dose-response relationship for any illness. Levels of *E. coli* were not found to correlate with illness. Dose-response relationships were seen for GI illness (faecal streptococci levels above 32/100ml) and acute febrile respiratory illness (AFRI – faecal streptococci levels above 60/100ml). The variability in FIO was taken into account when calculating the burden of disease attributable to recreational water exposure by combining the dose-response relationship with a probability density function describing the distribution of FIO. This allowed for both the mean and variance of the bacterial distribution to be taken into account.

In Europe, a randomised control trial was conducted at five freshwater sites in Germany (four lakes and one riverine site); sources of faecal contamination included treated and untreated municipal sewage, agricultural runoff and water fowl [(11)]. Relationships were demonstrated for three different definitions of GI illness and ENT and *E. coli*. Relative risk values depended on the definition of GI and ranged from 1.8 (95% CI 1.2-2.6) to 4.6 (95% CI 2.1-10.1).

Epibathe was a European-based study which was specifically designed to address the "*relative paucity of EU data describing the health effects of bathing in EU freshwaters and Mediterranean marine waters*" [(12)]. Eight separate randomised control trials were completed, four at different freshwater sites in Hungary and four at two different marine beaches in Spain. The results from these trials were analysed both separately and in combination with the existing data acquired, using the same methodology [(9, 11)]. The risk of GI illness was higher in bathers (compared to non-bathers) in both the Spanish and Hungarian studies, although not significantly so. Analysis of the combined data set (using meta-analysis), specifically the GI symptoms, suggested that ENT was the best predictor of illness in bathers using marine waters (combined data OR 1.38; 1.03-1.87) and that *E. coli* may be a better index of GI symptoms in bathers using freshwater (combined data OR 1.19; 0.88-1.62). Results from the freshwater studies, however, were not statistically significant and did not show a consistent exposure-response association (i.e. an incremental increase in illness with increasing FIO exposures).

The evidence from the European studies and other international research outputs (both published and in progress) was considered at an international expert meeting in 2009. The resulting output was an addendum to the 2003 Guidelines for safe recreational water environments [(8)], which concluded that no change was required to the current WHO water quality Guidelines [(7)].

The studies published from temperate locations since the update to the WHO Guidelines [(7, 8)] are summarised for GI illness (the most commonly reported outcome) in Table B2 [(13-23)]. It can be seen from this Table that much of the recent epidemiological research has shown no relationship between bather health and *E. coli* concentrations. Where a relationship has been suggested [(19, 20)], there is inadequate information to derive a dose-response relationship. In addition to the studies outlined in Table B2, a combined analysis of a number of studies has been performed. Skin symptoms in swimmers versus non-swimmers at FIO levels above and below the USEPA [(4)] recommended threshold levels were compared [(24)]. Twenty studies were analysed (nine freshwater, eleven marine) and statistically significant results were reported for ENT and *E. coli* for marine sites.

Table B2: Summary of epidemiological studies (2009-2017) conducted in temperate locations and relationships with *E. coli*

		Beaches (n)					
Country (Reference)	**Study type**	**Pt source**	**Non-pt source**	**Summary water quality (EC)**	**Overall GI effect***	**Relationship between GI & *E. coli***	**Comments**
Marine							
USA [13]	PC	1 (Int.)		Faecal coliforms measured but not reported	√/x	x	GI effects seen when the berm was open (point source discharge)
USA [14]	PC	-	1	GM (max) cfu/100ml: 13 (1,000)	√	x	
USA [15]	PC	1 (Int.)		GM faecal coliforms (max) cfu/100ml: 44 (>2,000)	√/x	x	Relationship seen when the SGD operating
USA [16]	LC		2	Faecal coliforms measured but no summary measures given	x	x	Beaches affected by urban runoff, winter study in surfers
Greece [17, 18]	PC		3	GM (95%ile) cfu/100ml Beach A: 2.2 (4.9) Beach B: 1.9 (10.8) Beach C: 1.6 (4.7)	√	x	Symptoms thought to be related to bather density
Denmark [19]	RC			FIO peak cfu/100ml based on modelled data 2010: *E. coli* 26,000 2011: *E. coli* <500	√	*E. coli* & GI OR not given (data shown graphically)	GI effect seen in 2010 vs 2011 participants and in 2010 water swallowers vs non-swallowers
Fresh water							
USA [20]	PC		1 (Predom)	Mean (max) cfu/100ml 95 (1,538)	√	*E. coli* & GI: AOR 7 (1.5-32) based on exposure to highest quartile of *E. coli*	
USA [21, 22]	PC	CAWS	GUW	Mean cfu/100ml *E. coli* CAWS: 582 GUW: 45	√	x	Limited-contact water recreation
Netherlands [23]	PC	2		Utrecht (U) – no data Amsterdam (Am) max cfu/100ml *E. coli*: 10,0000	U x Am √	Not determined GI & self-reported water swallowed	Amsterdam site subject to sewer flooding 2 days before the event

* Overall GI effect seen between bathers versus non-bathers PC: prospective cohort LC: longitudinal cohort RC: retrospective cohort GM: geometric mean 95%ile: 95th percentile
Int. intermittent SGD: submarine groundwater discharge Predom: predominantly CAWS: Chicago area waterways system GUW: general use waters

B4. Water quality analysis

Methods for the analysis of bathing water quality have, traditionally, been based on culture techniques, where the target bacteria in the water sample are grown using selective media and suitable incubation temperatures. Distinctive features, such as growth at 44 °C and expression of specific enzymes, are used for positive identification and results are presented as the number of target bacteria per volume of water (usually 100ml). As bacterial growth is required, culture techniques typically require at least 18 hours before the results are available and so there has been a move to develop alternative methods which can provide more rapid results.

The most commonly used molecular method is quantitative polymerase chain reaction (qPCR), which works through the *in vitro* amplification of specific segments of the genome (DNA or RNA) from the microorganism in question. To date, there are two related recreational water regulatory approved qPCR methods, both for ENT (Method 1611 [25] and Method 1609 [26]).

Key requirements for analytical methods are sensitivity (the ability to detect small numbers of the target organism) and specificity (the ability to detect only the target organism) and, in addition, methods need to be repeatable (within a laboratory) and reproducible (between laboratories). It is also useful to consider the complexity of the test (which will have implications for staff training), the need for specialised equipment, the cost-benefit analysis and the time required to get accurate results [1].

The BWD stipulated a choice of two (International Organization for Standardization (ISO) methods, based on culture techniques, although Member States can use alternative methods providing that the alternative method's equivalence to the reference method is demonstrated.

The ISO methods specified in the BWD for *E. coli* are ISO 9308-1 [27] and 9308-3 [28]; these reference methods are undated, and so it is mandatory to use the current edition of the method for compliance monitoring. 9308-1, however, was updated in 2014 (and the previous method withdrawn) and is only suitable for waters with low bacterial numbers, as background growth can interfere with the enumeration of *E. coli*. The current version of ISO 9308-1 is, thus, not applicable to all bathing waters [29]. ISO 9308-2: 2012 [30] was not available when the BWD was adopted. It is based on Colilert®-18 method (IDEXX) and has been validated for bathing water monitoring for *E. coli* in European marine and freshwater bathing sites and is currently in use [29].

B5. Bathing water profile

Bathing waters designated under the BWD require a bathing water profile (Annex III). This includes identification and assessment of pollution (and its causes) that could impact on both water quality and bather health. The profile is, principally, intended to lead to an understanding of the faecal sources and pollution routes impacting a site. This can be used to plan appropriate management measures and as a source of information to communicate bathing water quality information. There are a number of tools which may assist in aspects of conducting a bathing water profile, including detailed water quality studies, faecal source attribution (including microbial source tracking - MST) and quantitative microbial risk assessment (QMRA).

B5.1 MST

The idea behind MST is that genetic markers within certain faecal microbes are strongly associated with specific hosts (e.g. humans, livestock, dogs and gulls) and that certain identified attributes of

those microbes can be used as markers for faecal contamination from that host [31]. Table B3 lists some commonly used MST targets and their associated hosts that have been used for investigation of recreational water.

Table B3: MST targets and associated hosts

Human	Cow/ruminant/pig	Gull	Dog
Human viruses:	CowM2	Gull2	DogBac
Enterovirus - EV	CowM3	LeeSeaGull	BacCan
Adenovirus - AdV	BacCow	Gull4	
Norovirus (GI) - NovGI	BacR		
Norovirus (GII) - NoVGII	Rum2Bac		
Polyomavirus JC – PyV-JC	Bovine AdV - BAdV		
Polyomavirus BK – PyV-BK	Bovine PyV - BPyV		
HF183	Pig2Bac		
BacHum	Porcine AdV - PAdV		
HumM2			
Lachno2			
HB			

MST has been applied to a number of bathing waters and the techniques have been successfully used to guide beach management / remediation decisions, where targeted interventions have led to a reduction in beach FIO concentration [32-34].

As the presence of human faecal contamination seems to be necessary for an FIO dose-response relationship, the USEPA have developed MST markers, measured using qPCR, to facilitate the identification of human sewage [35]. Where sanitary survey and the use of MST markers shows that recreational waters are free from sewage and other faecal matter of concern (e.g. from ruminants), the USEPA allows for the setting of a site-specific FIO target level [4].

While the concept behind source tracking is conceptually clear, the application of techniques and interpretation of results is work in progress [36]. Ideally, source apportionment, using MST, would allow just that, the knowledge that (say) 15% of FIO are derived from human sources, 75% from gulls and up to 10% from dogs and other unspecified sources. Unfortunately, such quantification currently relies on a number of assumptions [36-38], which often are not fully met or are untested, including:

- host-specific markers are host-specific and do not cross react with other species;
- host-specific markers have similar environmental survival rates, fate and transport;
- the species of interest shed a similar amount of its host-specific markers;
- the FIO: marker relationship is similar between species and markers;
- each host-specific marker has a similar prevalence and proportional distribution among individuals within the species.

B5.2 QMRA

QMRA consists of four steps (hazard identification, exposure assessment, dose-response assessment and risk characterization), with data for each of the steps drawn from an appropriate mix of the published literature, site-specific measurements and clearly documented assumptions. The application of QMRA to recreational water can be used to investigate a range of different scenarios and management questions (in a hypothetical manner), and can be used to augment and complement epidemiological studies [39] and to improve routine bathing water monitoring and management [40]. Some of the questions posed by recent recreational water QMRAs include [39, 41-49]:

- What sources of faecal contamination are likely to represent the greatest risk of infection?
- What is the impact of mixed faecal contamination on illness risk and allowable levels of FIO?
- What pathogens are likely to cause the illness rates seen in an epidemiological study?
- What is the health impact of incidental contact recreation from freshwater receiving secondary treated (but non-disinfected) effluent?
- What is the risk of illness from specific pathogens potentially present in bathing waters (e.g. *Cryptosporidium*, *Giardia* and adenovirus)?
- What is the impact of storm water/wet weather on the risk of recreational water related GI illness?
- What concentrations of MST markers suggest a bather GI illness rate of 30/1000 swimmers?

The results of these studies highlight the importance of viruses as a key cause of recreational illness, provide support for the greater risk posed by human (and also bovine) faecal contamination, indicate the importance of rainfall in increasing incidence of illness and suggest possible reasons why epidemiological studies may not always find a relationship between FIO and swimmer health.

The use of QMRA within a regulatory framework is currently being trialled in California (USA) and Alberta (Canada) where, in the absence of human or bovine MST markers, QMRA can be used to develop site-specific faecal indicator levels using the approaches described by Schoen *et al*. [41].

B6. Prediction (for daily beach management decisions) and discounting

Where recreational water is subject to occasional and predictable deterioration (such as after rainfall) and where users can effectively be discouraged from entering the water during such periods (e.g. through signage/beach advisory notices), the WHO Guidelines [7] suggest that the classification may be upgraded to reflect the water quality that users are actually exposed to during periods not covered by 'advisory' signage, providing that there is accompanying explanatory material. Thus, results from water quality samples taken during this period can be discounted from the overall classification.

Modelling has been put forward as a means of facilitating the prediction of periods of poor water quality, enabling timely (near-real-time) and appropriate information to protect public health [8]. A number of model types have been investigated for use in recreational water quality prediction [e.g. 50-57] including Multiple Linear Regression (MLR), Artificial Neural Networks (ANN), decision tree and hydrodynamic modelling, with MLR being the most commonly applied for daily beach management decisions. To be useful management tools, predictive MLR models should achieve a high explained variance (R^2 value) possibly >60% with well documented control of multicollinearity [58]. Where this could not be achieved through simple black box modelling then further investigation of the contributing catchments and their human and animal microbial flux through budget studies, often termed quantitative microbial source apportionment - QMSA [59], was recommended [58]; possibly with the parallel application of more complex and process-based hydrodynamic modelling better to determine the linkage from the multitude of input fluxes to the impacted bathing water sites [60, 61].

Statistical models use observed 'associations' between impaired water quality and measurable environmental parameters in the antecedent period leading up to the prediction. Observed associations do not prove 'causation' between the environmental variable and the change in water quality. Causation and the implied physical connectivity can be investigated further through tracer studies using microbial (e.g. phages) and/or dye (e.g. Rhodamine WT) tracers. These are generally

used in conjunction with QMSA investigations to define flux from a multitude of FIO sources potentially impacting upon a bathing water location [62, 63]. Simple rainfall thresholds were investigated in the development of early UK prediction of bathing water quality [64] and, in some cases, can be effective. However, it is generally true that the drivers of FIO concentration in recreational waters are more complex than can be characterised by a single predictor. It is for this reason that the most common statistical model applied to bathing water prediction is an MLR model. These are commonly available in commercial software systems which allow for parametricity testing of the raw data to ensure the data are appropriate for the statistical approach employed.

Most of the black box statistical modelling systems in use today (e.g. the US Virtual Beach and Nowcast software, the UK and Portugal [64, 65]) predict the water quality on the bathing day through one, early morning, model run, on which any public advisories (warnings) are based.

The principal strength of the MLR approach is that it can be built using regulatory (FIO) data and archive data describing candidate predictor variables. Thus, it can be applied without the requirement for new microbial data acquisition in most cases. Its main weakness is the implicit assumption that water quality on the bathing day is characterised by a single sample and is constant. This assumption has been questioned [66, 67]. Indeed, recent investigation at two UK sites subject to intensive sampling (half hourly samples throughout the bathing day for 60 days during the bathing season) observed ten to 1000 fold variations in FIO, with significant diurnality at one site surveyed.

Although modelling costs (especially where data acquisition for dependent and predictor variables is required) are perceived to be high, model implementation has the potential to enhance the health of bathers and the chance of a beach complying with water quality standards (through discounting). It can also reduce the impacts on availability/use of the beach (with the associated impacts on tourism and local beach-side economies) and potentially provide significant cost savings as managers are not forced to seek to reduce FIO loading during peak events to see a rapid improvement in both public health protection and compliance.

B7. Classification

The current bathing water classification requires an assessment of both ENT and *E. coli*, as shown in Table B4, and is based on results from a four-year period (or three-year if agreed) and should consist of at least 16 samples (although an assessment of a newly identified bathing water can be based on results from a shorter period providing the requirements for the minimum number of samples has been met). Samples are taken, immediately before and then, at least monthly, throughout the bathing season.

Samples taken during short-term microbiological pollution (affecting the bathing water for normally no more than 72 hours) can be discounted as long as a number of requirements are met, these include ensuring that bathers are deterred from entering the water during that period, an additional sample is taken after the end of the pollution of the affected bathing water to replace a disregarded sample and a stipulation that no more than one sample per year or no more than 15% of samples from the assessment period (whichever is greater) fall into this category.

Table B4: EU Bathing Water Directive standards for recreational water and classification results for 2015 & 2016 [6, 68, 69]

Parameter	Excellent quality	Good quality	Sufficient	Poor	No classification^
Inland waters					
ENT (cfu/100ml)	200 (*)	400 (*)	330 (**)		
E. coli (cfu/100ml)	500 (*)	1000 (*)	900 (**)		
Coastal & transitional waters					
ENT (cfu/100ml)	100 (*)	200 (*)	185 (**)		
E. coli (cfu/100ml)	250 (*)	500 (*)	500 (**)		
Bathing water classification					
2016 classification (%)	85.5	8.4	2.4	1.4	2.3
2015 classification (%)	84.4	9.1	2.6	1.6	2.3

(*) based upon a 95-percentile evaluation (**) based upon a 90-percentile evaluation ^ quality classification not possible

It can be seen from Table B4 that the majority of EU bathing waters are classed as having excellent or good water quality, with less than 3% being 'sufficient' and less than 2% 'poor'. The percentage of both fresh and marine bathing waters achieving excellent quality (or complying with the guide values from the earlier Directive) has steadily been increasing although, overall, inland sites lag behind marine sites.

Preliminary results from a questionnaire survey of Member States suggests that, overall, the classification of marine waters is more likely to be driven by concentrations of ENT than *E. coli*. A check of the data available for the 2016 bathing season assessment, however, indicated that for most of the marine sites both parameters were important, whereas for the fresh water classification the principal driver is *E. coli*.

Results from the 2016 bathing season monitoring show that 516 bathing waters (336 coastal and 180 inland) are classed as sufficient. A questionnaire survey of Member States suggested that if the sufficient classification was removed it was likely that a number of these beaches would de-designated. This is likely to have not only immediate negative effects on the affected local communities, but also longer term negative consequences such as a further reduction in water quality following the removal of active beach management [70].

B8. Conclusions

The stated purpose of the BWD is "*to preserve, protect and improve the quality of the environment and to protect human health*" (Article 1). The focus in this fact sheet and WHO recommendations, however, is solely on health protection.

E. coli should be retained within the BWD. Although there is less epidemiological evidence (Section B3) for its inclusion (compared to ENT), it does seem to drive compliance at some designated bathing sites (often fresh water locations) and its continued use allows the examination of historical trends.

The four levels within the classification system (excellent, good, sufficient and poor) should be retained. The 'sufficient' category provides impetus for progressive improvement and it is possible that, if this category were removed, a significant number of the beaches may be de-designated (Section B7).

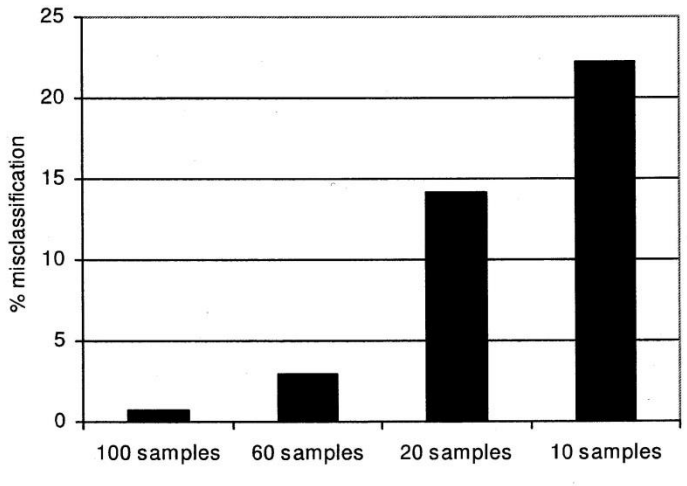
The classification system currently uses different assessment methods. 'Excellent' and 'Good' are based on a 95%ile value, while 'sufficient' is based on a 90%ile. This is confusing and difficult to explain to the public. It is recommended that all of the categories should be based on a 95%ile value; this retains consistency (for the most part) with the existing BWD and is in line with the WHO Guidelines approach. Based on a standard deviation of 0.8103 (derived from data from 11000 EU bathing waters and the value from which the WHO guidelines for marine water were calculated [71], the ***equivalent*** values for 'sufficient' are shown in Table B5. These values retain the same level of health protection as the current classification.

Table B5: Recommended changes to BWD sufficient classification for *E. coli*

	Sufficient (cfu/100ml)	
Water type	**Current (90%ile)**	**Amended (95%ile)**
Marine	≤500	≤993
Fresh	≤900	≤1789

Amended values based on a standard deviation 0.8103

The current minimum sample number for overall classification (16) leads to significant (i.e. 15-20%) misclassification of bathing water locations, as shown in Figure B1 [8]. In public health terms, using the epidemiological approach in WHO Guidelines for safe recreational water environments (2003), misclassification of a 'Poor' bathing water as 'Good' would imply an actual health risk of gastroenteritis exceeding 8.4% when the bathers should be assured of a health risk of 3-5% which could reduce confidence in the regulatory agencies concerned[7].



Based on hypothetical data, using parametric 95th percentile values and assuming a standard deviation of log_{10} values of 0.8

Figure B1: Misclassification rates in bathing waters [8]

This is considered unacceptable for a standard designed both:

(i) to communicate public health information to the general public; and

[7] Gastrointestinal Illness rates quoted come from the epidemiology used for intestinal enterococci in deriving the WHO (2003) guideline values.

(ii) to define the legal compliance of bathing water to EU regulators and Member States.

To avoid both the adverse health effects experienced by bathers of a poor bathing water misclassified as compliant, and the economic costs to local community businesses of a compliant bathing water being misclassified as poor, the ***annual*** minimum sample number collected in the bathing season in EU bathing waters should, therefore, be increased to 20 samples per site, with the overall classification being based on at least 80 samples collected over four years. In some cases (where locations have not undergone any major changes which are likely to change microbial levels), it may be appropriate to base the classification on more than four-years of data (in order to reach the new minimum sample numbers). It is noted that time will need to be allowed for the countries taking the current annual minimum number of samples (four) to adjust to the new sampling regime (20). Where significant infrastructure investments are made at an existing site (hopefully producing a 'step-change' improvement in water quality), it is expected that only sample data produced since the improvement (as required in the BWD) will be used: this will involve an unavoidable reduced sample number for interim compliance assessment.

Recreational water quality data is not always $\log_{10}$ normally distributed (although this tends to be the assumed position). The data from bathing water sites with at least 80 samples should be tested for $\log_{10}$ normality, using the Shapiro-Wilk test. Where $\log_{10}$ normality is demonstrated, the calculation method used in Annex II of the BWD can be used. Where data is not shown to be $\log_{10}$ normally distributed, the Hazen method of calculation should be used to calculate 95%ile values [(8)]. Log_{10} normality should be reviewed annually using the full data sequence to be used for compliance assessment. For sites without the required number of samples, it is suggested that it is assumed that the data is ***not*** $\log_{10}$ normally distributed (and thus the Hazen method should be used until enough samples have been analysed).

Accreditation determines the technical competence and integrity of organizations offering a range of services, including microbiological analysis. Sampling and sample analysis should be conducted by laboratories which are accredited for the methods being used. Detection levels can impact on the beach classification. Member States currently have to use the ISO methods specified in the BWD (or methods with demonstrated equivalency), the limits of determination (LoD) should be based on the specified test method (e.g. 3/100ml for membrane filtration and for some of the most probable number methods and 15/100ml for the microplate MPN method). Advice is available on appropriate dilution practices [(e.g.72)] and accredited laboratories should be able to consistently achieve these levels.

There is currently no ISO method for membrane filtration of *E. coli* which is suitable for bathing water use (although some Member States have made adaptations to the current method and earlier methods which have been approved on a country-by-country basis), this is an urgent research need. ISO 9308-1 should no longer be a BWD recommended method. ISO 9308-2 should be an approved method within the BWD.

A wide range of molecular methods have been developed (such as qPCR), however, it is not currently recommended that these be used for regulatory purposes in the BWD as dose-response data using these methods have not been obtained from European study sites and the methodology is not yet mainstream. If European-based epidemiological studies are conducted in the future, it is

suggested that qPCR methods for ENT and *E. coli* (and possibly enteric virus and MST markers) are included in the water quality microbial analysis suite to examine their possible suitability for European regulation.

Many recreational waters exhibit marked spatial and temporal variability. The BWD currently recommends that the monitoring point should be the location where most bathers are expected, or where the greatest risk of pollution is expected (according to the bathing water profile). In terms of spatial variability across the designated protected bathing area, it is suggested that the water quality should be representative of the whole bathing area (demonstrated within the bathing water profile); this could be confirmed by annual spatial/beach shoreline transect sampling. Temporal variability could be addressed by sampling at different times of the day, or taking a precautionary approach and sampling in the morning, when water quality is generally poorer [(73)], unless it is influenced by bather contamination.

Predictive modelling (Section B6) can provide information on daily beach management decisions (e.g. notification of the public that the water is not suitable for bathing). While their use is not currently widespread their potential contribution to public health is noted. Where they are employed, the model type and predictive variables used (e.g. rainfall) should be the choice of the Member States. The choices of models and methods of public information dissemination should be reported to the EC and the models employed should meet minimal requirements (including an explained variance of ***at least*** 50-60%) and the approach taken should be justifiable and auditable. The models should be optimized to predict the higher end microbial concentrations. The results should be used to inform the public, rather than for regulatory purposes, although where public 'informed-choice', at the time of predicted high results, can be demonstrated (e.g. through timely signage and electronic communication tools), they could also allow the discounting of water sample results taken at the time of the event (within current BWD specified allowances). Research is needed on the minimum number of data points required for model building and also whether prediction models could be used instead of a check sample to allow a return to use after a short-term pollution event.

Threshold values may provide guidance on site re-opening (i.e. de-warning), but they are likely to be site specific and, thus, it is felt that they are not amenable to regulatory use.

While methods such as MST and QMRA (Section B5) provide useful information for the bathing water profile, their use should be optional. It would be valuable to commission a detailed, state of the art, review to provide standardized information and advice on their practical application to Member States.

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C. Possible parameter - viral indicator

C1 Introduction

Neither *Escherichia coli* (*E. coli*) nor intestinal enterococci (ENT), i.e. the existing parameters specified by the European Union (EU) Bathing Water Directive (BWD), is considered ideal, especially because the principal pathogens of bathing-water-acquired illness resulting from faecal contamination are thought to be viral, rather than bacterial [1-3]. Recent advances in methodology for viral measurement in environmental samples (principally the use of molecular methods, and quantitative polymerase chain reaction [qPCR] in particular) have led to the suggestion that enteric viruses (such as adenovirus, enterovirus or norovirus) could be used in water quality assessment. Bacteriophages (viruses that infect bacteria) have also been suggested as possible viral indicators. A number of different bacteriophages have been suggested as possible candidate indicator organisms, but the majority of research has been conducted on coliphages (viruses that infect *E. coli*) and so the focus in this factsheet is on these organisms.

Human adenovirus (AdV): AdV are double-stranded DNA viruses and are members of the Adenoviridae family. They are associated with a range of diseases, primarily respiratory and gastrointestinal. In comparison to RNA viruses, they are resistant to environmental (ultraviolet) inactivation and have been shown to be prevalent worldwide and are shed, asymptomatically, for a protracted period by infected people. Although AdV have been found to be responsible for a number of recreational outbreaks of waterborne illness the majority of these (11/13) were in swimming pools [4].

Enterovirus (EV): EV are single-stranded RNA viruses, which are members of the Picornaviridae family. Human waterborne EV are divided into four species EV-A, -B, -C and –D and include different types of polioviruses, coxsackieviruses A and B, echoviruses and enteroviruses. Enterovirus can cause conjunctival, respiratory or gastrointestinal illness, but can also cause more serious diseases such as meningitis, paralysis, myocarditis or hand–foot-and-mouth diseases. They are shed from the gastrointestinal tract and the upper respiratory tract. In a review of 55 viral recreational water-related outbreaks, coxsackievirus and echovirus were found to be responsible for three and ten outbreaks, respectively. All three coxsackievirus outbreaks were from natural water bodies (two from lakes and one from seawater), four of the ten echovirus outbreaks were from natural water, with the remainder from swimming pools [4].

Norovirus (NoV): NoVs are single-stranded, non-enveloped RNA viruses belonging to the Caliciviridae family; they are the most common cause of viral gastroenteritis in humans. NoVs are genetically and antigenically diverse, with most of the strains relevant to human disease classed as being within genogroups I and II [5] (NoV GI and GII). In a review of viral recreational water outbreaks [4], 25 NoV outbreaks were identified (from a total of 55 outbreaks) of which 16 were associated with fresh waters (lakes - 14 and rivers - 2), with the other nine outbreaks being attributed to pools (7), a hot spring (1) and a fountain (1). More recently, in Scotland, an outbreak of NoV was reported in participants of an open water swimming event at Strathclyde Loch. An 85% attack rate was reported in swimmers [2]. An outbreak of NoV in Oregon in 2014 was attributed to a recreational lake, with people who swam in the lake being twice as likely to become ill compared

with those who did not swim [3]. A number of suspected bathing water outbreaks in Finland were investigated [1] and it was found that NoV was the main causative agent in the eight confirmed outbreaks (with NoV GI and GII isolated from the bathing water in two of the outbreaks). NoV infection has been shown to have a marked seasonal pattern and, in the northern hemisphere at least, has been described as a wintertime phenomenon [6]. This pattern has an impact on the occurrence of NoV in sewage and environmental water samples and reduces its value as a potential indicator.

Coliphages: Coliphages are split into two groups:

- somatic coliphages, which infect host coliform bacteria via their cell wall (somatic) receptors; and
- F-specific (also referred to as F+ or male-specific) coliphages, which infect bacteria through the sex- or F-pili. There are DNA and RNA F-specific coliphages.

F-specific RNA coliphages are morphologically similar to EV and NoV, while somatic coliphages are more diverse, but generally larger and some of them are similar in size to AdV [7]. Coliphages are present in sewage, as well as many animal faecal sources, and have been isolated from both fresh and marine recreational waters, although generally in low numbers.

C2. Current situation

There are currently no viral indicators as parameters in any of the major recreational water regulations, although the United States Environmental Protection Agency (USEPA) has completed a recent review of coliphages [7] and is in the process of developing a Recreational Water Quality Criteria, which is anticipated to be available in 2018. The USEPA regulatory interest in coliphages is driven by their consistently high presence in raw sewage and the fact that they may provide (in at least some cases) a useful index of enteric virus reduction by sewage treatment processes, including loss of infectivity following disinfection.

C3. Occurrence of enteric viruses and coliphages in European recreational water

The literature on the occurrence of selected viruses and coliphages in European recreational water [8-15] is summarised in Table C1. The largest European studies are also outlined in greater detail.

The EU project Virobathe (https://cordis.europa.eu/result/rcn/51774_en.html) examined the methodological feasibility of including viral parameters (specifically AdV and NoV) in a future update to the BWD. The work compared methods for the detection (presence/absence) of AdV and NoV and derived a combined concentration and detection technique to provide a reproducible system of testing recreational waters for these viruses. Fifteen laboratories in nine countries were involved in the surveillance phase of the project. Over 1400 samples (marine n=482; freshwater n=928) were taken from recreational water sites; 43% of the freshwater samples and 31% of the marine samples were positive for viruses. AdV were detected more frequently than NoV. Subsets of viral positive samples were also subjected to AdV infectivity determination and AdV quantification using qPCR. From the 51 marine samples tested, 47% were found to contain infectious AdV [8]. Lower infectivity rates were seen in the freshwater samples (n=226, infectivity - 20%). AdV from 132 fresh and marine water samples (which had tested positive for AdV by nested PCR) were quantified [16]. Overall, the

mean value was 32,000 genome or gene copies (GC)/litre, higher mean concentrations were reported from the marine samples (91,000 GC/litre) compared to the freshwater samples (560 GC/litre). Comparison of the viral results with FIO levels set by the EU BWD suggested that over 50% of samples that were relatively clean and which exhibited 'good' water quality (as defined by the EU BWD) could, nevertheless, be positive for AdV and NoV [8]. The results for AdV (n=290) and the corresponding sample FIO concentrations were examined [17]. Statistically significant trends in the proportion of AdV positive results with increasing FIO concentrations in fresh (but not marine) water samples were seen. The proportion of AdV positive results increased consistently from below 50% in the first quartile FIO categories to over 79% in the final FIO quartile groups. Significant trends were also seen when categorizing FIO concentrations into 0.5 $\log_{10}$ interval groups.

Table C1: Virus occurrence and concentration data in European recreational water

Virus	Country	N	Occurrence[a]	Viral concentration
Fresh water				
AdV	Various European[b]	928	41%	Presence/absence
	Hungary	37	51%	Max: 1,020 GC/l
	Hungary	129	98%	GM: 5,653 GC/l
	Sweden	137	11%	GM: 60 GC/l
	Greece	70	26%	GM: 199 GC/l
	Spain	73	79%	GM: 474 GC/l
	Finland	38	11%	Max: 3.4×10^7GC/l
EV	Hungary	42	12%	Presence/absence
NoV GII	Hungary	129	40%	GM: 402 GC/l
	Sweden	136	6%	GM: 122 GC/l
	Greece	70	13%	GM: 187 GC/l
	Spain	73	71%	GM: 145 GC/l
NoV GI/GII	Various European	928	6.3%	Presence/absence
	Hungary	42	14%	Presence/absence
Somatic coliphage	Germany			Max: 37,800 pfu/l
Marine and brackish water				
AdV	Various European	482	27%	Presence/absence
	Sweden	68	13%	GM: 59 GC/l
	Greece	70	31%	GM: 232 GC/l
	Spain	32	66%	GM: 474 GC/l
	Finland	12	17%	Max: 1.3×10^7 GC/l
NoV GI	Portugal	22	27%	Presence/absence
NoV GII	Sweden	68	9%	GM: 125 GC/l
	Greece	70	24%	GM: 243 GC/l
	Spain	32	19%	GM: 145 GC/l
NoV GI/GII	Various European	482	16%	Presence/absence
Somatic coliphage	Spain	20	95%	Max: 122,400 pfu/l
	Spain	806	73%	95th percentile*: 44,400 pfu/l
F-specific coliphages	Spain	20	20%	Max: 840 pfu/l
	Spain	429	26%	95th percentile*: 910 pfu/l

[a] % of samples positive for the virus Max: maximum concentration GM: geometric mean GC/l: genome copies/litre pfu/l: plaque forming units/litre * 95th percentile value of the location with the highest concentration

[b] Cyprus, France, Germany, Italy, Netherlands, Poland, Portugal, Spain, UK

The Viroclime project built on the work of Virobathe and measured virological water quality over an 18-month period using qPCR (AdV and NoV) at four European sites in Spain, Greece, Sweden and Hungary [10]. The highest AdV values were 3×10^6 GC/litre in river water samples and 5×10^4 GC/litre in marine samples. Some statistically significant correlations between the key virus parameters and FIO were seen, but the highest level of explained variance (R^2) was only 0.228, which indicates that

(in this instance) the $\log_{10}$ *E. coli* concentration only explained 22.8% of the variance in AdV in the waters tested. It is expected that in order to be acceptable from a regulatory perspective, explained variance levels would need to exceed at least 50%.

While the European data for coliphages in recreational water is quite limited, there is a suggestion from Table C1 that (at least for marine and brackish water) somatic coliphages seem to predominate and are present in greater number than F-specific coliphages. This is supported by a recent global review [18], where it was shown that the mean level of somatic coliphages in fresh and marine samples was 15,130 pfu/l and 460 pfu/l, respectively; compared to F-specific coliphages where the calculated mean levels were 1,000 pfu/l and 80 pfu/l in fresh and marine samples.

C4. Epidemiological data

Epidemiological studies are used to evaluate illness resulting from exposure to contaminants and/or activities and have been used to inform recreational water quality guidelines and regulations. The studies typically evaluate the levels of illness in swimmers (or other water recreators) and non-swimmers and relate the illness rates to the exposure (usually characterised by levels of FIO). Results are typically expressed as odds ratios (OR) or other types of relative risks (RR) and there is a statistically significant increase in risk between the groups if the lower 95% confidence interval (95% CI) is greater than one (approximately corresponding to a p-value of <0.05). Studies usually examine a range of possible illnesses, such as gastrointestinal (GI) illness, respiratory problems, eye, ear and skin symptoms. The exact definitions of the illnesses and symptoms vary between studies.

C4.1 Enteric viruses

Few epidemiological studies have looked for, or found, a relationship between health outcomes and enteric viruses. Results of an analysis of freshwater samples [19] archived from a 2010 epidemiological study conducted in the USA [20] in relation to the swimmer-reported gastrointestinal illness have been reported. Twenty-three samples were analysed by qPCR, for four human viruses (AdV, EV, NoV GI, NoV GII) and four bacterial markers and were paired with the results from human exposure data (600 swimmers). AdV was the most frequently identified virus and was reported in 35% of the samples. None of the qPCR measurements showed a significant association with illness in single microorganism models using univariate or multivariate logistic regression. They did, however, report a significant positive association between exposure to AdV and diarrhoea and also GI illness (AOR 1.6; 95% CI 1.1-2.3 and AOR 1.5; 95% CI 1.0-2.2 respectively) when culturable *E. coli* concentrations were included in multivariate models. The authors suggest that the study "*demonstrates the predictive potential of an integrative, multi-microbial approach for estimating recreational waterborne disease risk from viral and bacterial indicators.*"

C4.2 Coliphages

Epidemiological studies which have examined coliphages are summarised in Table C2 (which is based on a review conducted by the USEPA [7]).

Table C2: Summary of epidemiological studies using coliphages as FIOs

Water type	Coliphages evaluated	Results
Marine	Somatic coliphages F-specific phages	Very low levels of coliphages detected, no relationship seen with health outcomes [21].
	Somatic coliphages F-specific phages	Despite low concentrations of F-specific phages a significant association was seen for some measures of GI illness and the indicator [22]; thus the AOR for one of the HCGI definitions was 1.25 (95% CI: 1.13-1.82).
	F-specific phages	The AOR was significantly higher in swimmers, compared to non-swimmers on days when F-specific phages were detected. An increase in GI illness in swimmers was seen for a $\log_{10}$ increase in coliphages, but this was not statistically significant [23].
	Somatic coliphages F-specific phages	F-specific phages were not detected. There was no statistically significant correlation with health outcomes and somatic coliphages [24].
	F-specific phages	F-specific phages measured using EPA method 1602 had a stronger association with GI illness than ENT, although the association was not statistically significant [25].
	Somatic coliphages F-specific phages	Pooled analysis of a number of studies (including [22, 23]). Under human impacted conditions, the presence of coliphage was associated with an increase in GI illness (although this was not statistically significant). Under human impacted conditions there was a statistically significant relation between GI illness and coliphage when ENT was greater than 35 cfu/100ml [26].
Fresh	F-specific RNA phages	Significant association between GI illness and measured phages [27]. In comparison to a reference level of 10-30 pfu/100ml, the RR for GI illness at 260 to 320 pfu/100ml was 2.6 (95% CI: 1.3-5.2) and at 690 to 3080 pfu/100ml the RR was 2.8 (95% CI: 1.3-6.0).
	F-specific RNA phages	No relationship between coliphages and health outcome was observed [28].
	Somatic coliphages	Significant increased risk of GI illness in bathers compared to non-bathers when somatic coliphages were above 10 pfu/100ml [13].

GI: gastrointestinal HCGI: highly credible gastrointestinal illness AOR: adjusted odds ratio RR: relative risk CI: confidence interval cfu: colony forming units pfu: plaque forming units

C5. Analysis

Key requirements for analytical methods are sensitivity (the ability to detect small numbers of the target organism) and specificity (the ability to detect only the target organism) and, in addition, methods need to be repeatable (within a laboratory) and reproducible (between laboratories). It is also useful to consider the complexity of the test (which will have implications for staff training), the need for specialised equipment, the cost-benefit analysis and the time required to get accurate results [29].

C5.1 Enteric viruses

Although numerous research methods for the concentration and detection of enteric viruses in water have been utilised there are, currently, few standardized methods for the analysis of enteric viruses from water samples. As viruses are typically at relatively low levels in environmental water samples effective methods for concentration and sensitive detection methods are required.

Concentration methods are often based on a two-step adsorption-elution process using membranes, filters or matrixes, such as glass wool, although it is noted that these can be cumbersome and make the simultaneous processing of a large number of samples difficult. Although some viruses can be detected in water samples by cell culture (plaque assay), detection is now mainly done using

molecular methods (e.g. USEPA Method 1615 [30]) and all of the studies reporting on occurrence in Table C1 used PCR techniques.

In Europe, the Virobathe study aimed to produce robust, rapid and cost-efficient methods for routine compliance monitoring of enteric viruses in recreational waters. In order for virological water quality to be assessed on a comparable basis two methods (one for concentration of viruses from freshwater samples and one for marine samples) were employed by all the participating groups. Mean (range) AdV recovery, across all the laboratories, from spiked samples was 57% (34-78%) from freshwater using glass wool followed by elution with beef extract and 35% (22-44%) from artificial seawater using membrane filtration and skimmed milk elution. Sensitivity (based on the percentage of correctly identified positive samples) was 77% for freshwater and 89% for seawater, while the specificity (based on the percentage of correctly identified negative samples) was between 96 – 99% [8]. In addition, a simple one-step protocol for viral concentration, based on organic flocculation, was developed which has been used successfully in subsequent European studies [31, 32].

C5.2 Coliphages

Bacteriophages can be detected using a number of methods, with infectious bacteriophages typically being detected by the effects (especially lysis) they have on the host bacteria they infect. Bacteriophages are enumerated by direct quantitative plaque assays (with their concentration typically expressed as plaque forming units, or as most probable number). The most important factor in defining a method for the detection of a given bacteriophage (or group of bacteriophages) is the bacterial host strain. Standardised methods (e.g. USEPA and ISO methods) are available for both somatic and F-specific coliphages.

C6. Discussion

Although a number of enteric viruses (NoV in particular) clearly have a health basis, in terms of their use as a regulatory parameter they do not, currently, meet many of the other requirements.

- Viral pathogen presence often reflects the infection rate and associated viral shedding in the contributing population. Thus, the absence of key pathogenic viruses (e.g. NoV) cannot always be taken to infer a lack of human faecal connectivity to the bathing site and, for this reason, they may not represent as good a measure of risk of future pathogen presence as the existing bacterial FIOs which (in temperate climates) strongly indicate faecal connectivity to the bathing water. Other candidate viral parameters (e.g. JC polyomavirus) have more consistent shedding patterns but lack epidemiological evidence to support a standard or criteria for their use.
- Concentrations in recreational waters are often very low, meaning that detection is neither straightforward nor conservative.
- Many of the viral detection methods rely on qPCR methods, which are currently costly and enumerate non-infectious and infectious virions, making it unclear what the results mean in terms of health risks. This may present a particular problem where terminal disinfection is applied to secondary treated effluents and the qPCR signal from target viral pathogen genetic markers is not attenuated through the disinfection used at the plant, although recent research on capsid-integrity qPCR may reduce this uncertainty [33].
- There is insufficient epidemiological evidence for enteric viruses to allow the derivation of regulatory values. While there is more epidemiology available for coliphages and the evidence has been described as "*suggestive of a potential relationship between coliphages and human health*" [7] overall they lack consistency and do not provide a clear exposure-response relationship.

C7. Conclusions

While many recreational water illnesses are thought to be viral in nature, evidence does not currently support inclusion of a viral indicator (e.g. coliphage) or pathogen (e.g. norovirus and/or adenovirus) within regulations, as there are insufficient epidemiological data to allow derivation of regulatory levels.

Possible research needs include identification of suitable candidate organisms (e.g. a human enteric virus with consistent shedding patterns) and the development of standard methods which are suitably sensitive and can be consistently applied across different laboratories. It is also suggested that if additional European epidemiological studies are conducted that coliphages and selected human enteric viruses are included in the water quality microbial analysis suite to investigate if dose-response relationships (between viral water quality and bather health) can be demonstrated.

Viruses have a valuable role to play in MST investigations [34] and also QMRA which, it is suggested, should be considered more widely in bathing water profiling.

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D. Exploration of a harmful algal bloom parameter

D1 Introduction

There are a number of microalgae, whose rapid proliferation can result in the phenomenon known as a harmful algal bloom (HAB). Blooms can result in reduced water transparency, discoloured water and scum formation and, particularly during the bloom breakdown, there may also be aesthetic problems (such as smell) [(1)]. The creation of low oxygen (hypoxic) conditions can result in plant and animal die-off. Blooms are often caused by toxin-producing species although they can also be caused by non-toxic species [(2)]. Contact with the bloom may cause a number of negative health impacts, which may be associated with the known toxins or may result from direct contact with the cells [(3)]. Exposure to HABs from bathing water can be via dermal contact, inhalation of aerosols as well as ingestion of water or scum material; with ingestion being the key cause for concern. Ingestion of bloom material has been responsible for a number of animal deaths [(e.g. 4)] and some of the HAB toxins are highly potent.

In marine waters, dinoflagellates and diatoms are primarily responsible for HABs; while in freshwater it is cyanobacteria. These organisms are a natural part of the environment but under certain conditions (particularly when there is an overabundance of nutrients) their rapid growth can result in HABs.

Globally, the principal health concerns from marine HABs derive from the consumption of seafood (shellfish and fish) which concentrate toxins from the bloom. Depending upon the toxin type these can result in syndromes such as paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP) and ciguatera fish poisoning (CFP) [(1)]. Information on marine HABs is stored in the Harmful Algal Events Dataset (HAEDAT) database. During the 35-year period between 1980 and 2015 there were 3037 events in total [(2)], the vast majority of which were related to food poisoning.

There has been much speculation [(5, 6)] about the likely impact of climate change on the incidence of HABs (e.g. could the increasing water temperature and the increasing severe weather potentially have opposing effects on phytoplankton growth [(7)]). What is clear, however, is that many of the factors linked to climate change, such as warmer conditions, more extreme precipitation (causing increased erosion and, thus, nutrient input), rising carbon dioxide (CO_2) levels, increased (and earlier) thermal stratification, drought, salinization and lower pH in waterbodies are also linked to HAB frequency, HAB species composition, duration and distribution [(8, 9)].

From a recreational viewpoint it is HABs caused by cyanobacteria (primarily, but not exclusively, in freshwaters) that are the main cause for concern in water bodies used for recreation in the European Union (EU) and thus these organisms are the main focus. One reason for this is that the mechanism of cell concentration that leads to high toxin concentrations: for most marine non-cyanobacterial HABs this accumulation is in seafood (especially shellfish) and exposure is through its consumption, while for cyanobacteria it is the formation of surface scums or very high cell density in highly eutrophic shallow water bodies. The same mechanism applies to the most relevant cyanobacterial taxon (*Nodularia spumigena*) in marine and brackish waters. The epidemiology and

case study reports (published since 2003) and a number of recently published literature reviews (e.g. [2, 3, 9-14]) have formed the basis of this fact sheet.

D2. Current situation

HAB-causing organisms are not currently assessed as part of the EU Bathing Water Directive (BWD) bathing water assessment and classification; they are, however, specifically mentioned in Articles 8 (cyanobacteria) and 9 (marine phytoplankton) as a requirement to consider them as part of the bathing water profile. Where a bathing water profile identifies that there is a potential for proliferation further investigations (including appropriate monitoring) are required to determine the likely health risks. When health risks are identified: "*adequate management measures shall be taken, including information to the public*". For cyanobacteria, the requirement is that the measures shall be taken "*immediately to prevent exposure*". Thus, the BWD does not define specific actions to be taken, but it does indicate their required outcome [15].

In their 2003 publication [1] (based on the relatively limited geographical range of marine toxic algae/cyanobacteria), WHO did not recommend specific guideline values for marine HAB-forming organisms, although it was noted that "*authorities should be aware of the potential hazard and act accordingly*". Guidelines were, however, suggested for fresh water cyanobacteria in recreational waters [16] and these were based on the probability of health effects in relation to cyanobacterial level and chlorophyll-a concentrations. The guideline values were accompanied by a commentary on the risks and also suggested management actions. It was suggested that the approach should address the presence of cyanobacteria "*because it is as yet unclear whether all important cyanotoxins have been identified*"; this remains true today. The point was also made that many of the health outcomes observed after recreational exposure (especially skin and mucous membrane irritation) are probably related to cyanobacterial substances other than the well-known toxins (see Section D3). The guidance is currently under review (close to completion) and is expected to retain three levels and provide advice on recommended management actions [13].

Many of the European country-level standards are based on a similar alert level approach, with trigger values based on a measure of cyanobacterial bloom intensity, using parameters such as cell number, biovolume or pigment concentration (with phycocyanin being an accessory pigment to chlorophyll-a in cyanobacteria). The different alert levels typically define responses (such as increased monitoring) or specific interventions, such as advising against recreation [15]. This approach reflects the fact that the harmful effects of cyanobacterial HABS can be caused by a range of different possible toxins and bioactive substances.

The WHO (Chemical Group Meeting, March 2017) is currently working on drinking-water toxin-based guidelines for a number of cyanotoxins including microcystin (MC) and cylindrospermopsin (CYN). These will be based on animal toxicity studies and will be used as a starting point for the development of recreational water guideline values.

D3. Occurrence

This section provides a 'flavour' of occurrence, as a detailed review of the literature is beyond the scope of this fact sheet.

The following cyanobacterial genera (that include toxic species or strains) have been reported from countries in Europe [10]: *Anabaena*, *Aphanizomenon*, *Aphanocapsa*, *Aphanothece*,

Cylindrospermopsis, *Dolichospermum*, *Gloetrichia*, *Microcystis*, *Nodularia*, *Oscillatoria*, *Phormidium*, *Planktothrix*, *Raphidiopsis*, *Tychonema* and *Woronichinia*. In Europe, cyanobacterial dominance is greatest during the summer months, which coincides with the greatest demand for recreational water use [13]. A wide range of toxin concentrations have been reported, with the greatest concentrations typically seen in thick scums.

In the Mediterranean, blooms of *Ostreopsis* (tropical benthic dinoflagellates) have been reported since 2003, these have often been followed by reports of mild self-limiting respiratory and skin irritation in people exposed to seawater or aerosolized sea spray [17]. In France there is an active *Ostreopsis* surveillance network which encompasses the French Mediterranean during the bathing season. Alerts are triggered by either the detection of two patients with possible *Ostreopsis* clinical symptoms or when routine analysis samples exceed 30,000 *Ostreopsis* cells. Between 2006 and 2009 there were nine reported blooms, five of which resulted in reported clinical symptoms. During 2017, there were a number of reported blooms but, as a result of beach closures, no reported symptoms [18].

Examination of the harmful algae event database (haedat.iode.org), which contains information on marine algal blooms, showed that in 2016, there were five reported incidences of marine cyanobacterial toxin effects in Europe; three from Sweden involving *Aphanizomenon flos-aquae* (no toxicity testing performed) and two from Poland involving nodularins (maximum concentration 258 µg/l) and *Nodularin spumigena*, with one incident leading to the closure of ten beaches for a week in July (http://haedat.iode.org/viewEvent.php?eventID=5492).

D4. Health impacts

HABs have been associated with a range of health impacts, although it is their potential toxin content that is the principal cause for concern. Human deaths have been associated with the consumption of shellfish containing HAB-derived toxins [e.g. 19] and animal deaths have been attributed to the consumption of cyanobacterial bloom contaminated recreational water [e.g. 4, 14, 20]. No human fatalities, however, have been unequivocally linked to cyanotoxin ingestion during recreational water activities [16].

D4.1 Toxins

Cyanotoxins (Table D1) are often categorised according to their target tissues (e.g. hepatotoxins, neurotoxins etc.). Microcystins are the cyanotoxins most commonly reported in health-relevant concentrations [21]. Some cyanotoxins (such as microcystins) occur as a large variety of structural variants. The variants have differing levels of toxicity and methods of analysis vary in their ability to detect different types [22].

As noted in the WHO Guidelines [16], toxicity is not a trait specific for certain species, but most species comprise both toxic and non-toxic strains. It is not possible to distinguish between the toxic and non-toxic strains by observation and even toxic strains do not always produce toxins.

Table D1: Toxins produced by cyanobacteria (adapted from [2])

Toxins	Most common cyanobacteria genera producing toxins	Comments
Microcystins (MC)	*Microcystis* *Anabaena* *Dolichopermun* *Anabaenopsis* *Aphanizomenon* *Plankothrix* *Oscillatoria* *Phormidium*	MC are the most frequently occurring and widespread cyanobacterial toxins. Animal deaths linked to MC have been reported (e.g. 20)
Nodularin (NOD)	*Nodularia* *Nostoc*	Predominantly occur in brackish water [23] but are also present in benthic freshwater organisms [24]. Found in the Baltic Sea.
Cylindrospermopsin (CYN)	*Cylindrospermopsis* *Anabaena* *Dolichospermum* *Aphanizomenon* *Rhaphidiopsis* *Oscillatoria* *Lyngbya* *Umezakia*	A number of the species producing CYN (e.g. *C. raciborskii*) do not form visible surface scums, with the highest cyanobacteria cell concentration being below the water surface [3]. Dissolved CYN concentrations are generally higher than intracellular ones [10]. *Aphanizomenon* spp. are the most important CYN producers in Europe [10].
Anatoxins (ATX)	*Anabaena* *Aphanizomenon* *Dolichospermum* *Oscillatoria* *Tychonema* *Pseudanabaena*	Dog, livestock and waterfowl deaths have been attributed to ATX poisoning [14]. ATX poisoning in dogs is often associated with benthic cyanobacteria.
Saxitoxins (STX)	*Anabaena* *Dolichospermum* *Aphanizomenon* *Cylindrospermopsis* *Lyngbya* *Rhaphidiopsis*	Some animal deaths have been attributed to STX, but no European cases have been reported [14]. STX are most commonly associated with PSP after consumption of marine seafood [23]
Unknown substances	*Synechococcus* *Microcystis* *Anacystis* *Oscillatoria* *Schizothrix* *Anabaena*	Skin and eye irritation, headache, allergy symptoms, asthma and fever.

While the toxicity of some cyanotoxins have been demonstrated in animal testing conducted in the laboratory (for recent reviews see [10, 13]), evidence of human health impacts definitively linked to recreational water cyanobacterial exposure (e.g. from case/outbreak reports and epidemiological studies) is harder to obtain, especially as some of the symptoms observed could also be caused by pathogens, but tests to exclude these causes are rarely performed. However, the evidence from animal testing can be used to assess concentrations likely to be hazardous to human health.

In addition to direct exposure via actual water contact, cyanotoxins can, under some circumstances, be aerosolized through a bubble-bursting process. When this happens, cyanobacteria and cyanotoxins are ejected and carried into the air in the resulting droplets [25]. In addition to such true aerosols, exposure to cells carried in spray (e.g. through water skiing) is also possible.

Reports published since 2003 (the date of the WHO Guidelines) are covered briefly, below.

D4.2 Case and outbreak reports and anecdotal evidence

Marine

The majority of reports of health impacts from exposure to marine HABs are from tropical waters (and so are not covered here). Respiratory and gastrointestinal symptoms have been reported following exposure to *Karenia brevis* (a marine dinoflagellate which causes 'Florida red tides' [26, 27]) and skin lesions have resulted from exposure to the marine cyanobacteria *Lyngbya majuscula* [28] (now known as *Moorea producens*).

In Italy, a two-year syndromic surveillance study found more than 200 cases of respiratory syndrome, 20% of which required hospitalisation, in people who spent time near or on beaches during the presence of *Ostreopsis ovata* algal blooms [29]. The most frequent symptoms were fever, sore throat, cough and shortness of breath. The mean onset of symptoms was 4½ hours after the beginning of exposure.

Fresh

A review of case studies and anecdotal reports of health impacts from recreational exposure to freshwater cyanobacteria (dating back to 1949) was published in 2006 [30]. It was reported that hay fever-like symptoms, itchy skin rashes and GI symptoms were the most frequently reported outcomes. In a small number of cases more serious symptoms were reported, including severe headache, pneumonia, fever, muscle pain, vertigo and blistering of the mouth. There was also a report of a fatality in a teenager linked to exposure to anatoxin-a resulting from boisterous play in a scum-covered pond containing an *Anabaena flos-aquae* bloom. Although the coroner attributed the death to ATX, questions have been raised about the analytical methods used at the time and also the time of onset to the symptoms [13].

In Argentina, a young jet-skier was exposed to an intense bloom of *Microcystis* spp. with a reported MC concentration of almost 50 µg/l (MC-LR). Illness (fever, nausea, abdominal pain and muscle weakness) was experienced a few hours after exposure. He was later hospitalized for acute respiratory symptoms, requiring artificial ventilation. In the third stage, the patient exhibited liver damage. Despite the severity of the symptoms, complete recovery (after 20 days) was reported [31]. While, MC was detected in the water and liver damage was reported, the initial response was pulmonary toxicity, which is not typical of MC poisoning and it is possible that the symptoms may have been due to an unknown algal toxin.

In the USA, 11 recreational water HAB-associated outbreaks of illness were reported during the two year period between 2009 and 2010 [32]. The criteria for a HAB-associated outbreak were the linkage (epidemiologically) of two of more people using a recreational water and the presence of an algal bloom noted by state health or environmental investigators. Health effects included skin, GI, respiratory and neurological signs (such as confusion and tingling) and symptoms. All the outbreaks

occurred at freshwater lakes during the summer months (June to August) and in each case water contact and/or ingestion was reported. Where onset time (i.e. time from reported exposure to time of reported symptoms) was available, the median time (based on 27 people) ranged from half a day to two days. In eight of the 11 outbreaks water was tested for and found to contain one or more cyanotoxins. It should be noted, however, that other possible causes of symptoms (e.g. faecal contamination) were not ruled out.

Clinical studies indicate that some freshwater cyanobacteria can give rise to hypersensitivity reactions in some people [33, 34].

D4.3 Epidemiological studies

Marine

The majority of epidemiological studies which considered marine HABs that have been published since 2003 relate to tropical waters (e.g. [35 - 37]) and so are not covered here.

In Spain, a small-scale cohort study looked at the health impacts related to an *Ostreopsis ovata* bloom in the Mediterranean Sea, close to Barcelona [38]. The cohort (16 people working at an indoor-outdoor restaurant 10m from a bloom hotspot) were followed using a daily diary sheet from June to November, with bloom sampling being conducted in parallel to the health data acquisition. During the monitoring period, 13 of the 16 participants reported at least one of the bloom-related symptoms (e.g. eye and nose irritation, runny nose and general malaise). The health effects were found to be the greatest during a short period which corresponded to the transition from exponential growth to the stationary phase of the bloom. There were no clear patterns in landward wind during the greatest symptom reporting.

Fresh

Recent freshwater epidemiological studies (published since 2003) are summarised in Table D2. It shows that different study groups took quite different approaches. Each study considered other indicators of water quality (such as those related to faecal contamination) to, at least, try to ensure that any health symptoms were correctly attributed to cyanobacterial bloom exposure.

In many cases, studies are hampered by ethical concerns (given the potential exposure to very potent toxins) and/or the transient and unpredictable nature of a cyanobacterial bloom. Generally, levels of cyanobacterial exposure are often poorly characterised and few studies found statistically significant health differences.

Table D2: Fresh water cyanobacterial epidemiological studies (published since 2003)

Location (reference)	Exposure mechanism	Study size Study group	Cyanotoxin measured/ detected (max)	Comments
Australia & USA [39]	Immersion	1331 Water-contact related activity	MC (12 µg/l) CYN (2 µg/l) ATX*(1 µg/l) STX** - ND	Increased reporting of mild respiratory symptoms and any symptom (3 days post-exposure) to CB cell surface area >12 mm^2/ml vs <2.4 mm^2/ml. Respiratory symptoms OR 2.1 (1.1-4.0). Toxins were infrequently detected.
USA [40]	Aerosol exposure and/or immersion	97 recreators at a bloom affected lake & 7 using a bloom-free lake	MC (5 µg/l – water) MC (<0.1 ng/m^3 – air)	No statistically significant health impacts seen. Study was conducted within a week of detecting 10 µg/l MC. Toxigenic CB ranged between 54,000 to 144,000 cells/ml.
USA [41]	Aerosol exposure and/or immersion	81 recreators planning activities on target lakes & 7 using the control lake	Total MC (>350 µg/l – water). MC 0.4 ng/m^3 – personal air sample)	No statistically significant health impacts were reported. A range of personal exposure measures were used including personal air samplers, nasal swabs and blood tests.
Canada [42]	Drinking water supplies and/or recreational exposure.	466 residents living close to lakes affected by CB	MC (0.02 – 773 µg/l – depending upon the site). 10% of samples from shore locations at one of the lakes had MC concentrations >20 µg/l.	Data on symptoms and activities collected via an individual daily journal. CB cell counts ranged from 7 to >10^6 cells/ml. Some suggestion that GI symptoms were associated with recreational water contact with CB-containing lakes. It was noted that residents avoided full contact activities during blooms.

OR – odds ratio * - measured in USA only ** - measured in Australia only CB – cyanobacteria

D5. Water quality analysis and toxin detection

A detailed examination of the different methods and techniques is beyond the scope of this fact sheet (and a general introduction to many of the methods may be found in the following references [43 - 46]). However, a brief introduction is given to the various biological, biochemical and physicochemical methods and approaches that can be used to monitor cyanobacterial blooms and detect cyanobacterial toxins. While some of the methods can suggest that a bloom has 'toxic potential' (e.g. the presence of a cyanobacterial scum and high numbers of a potentially toxic strain), because toxigenic and nontoxic strains (which are morphologically indistinguishable) often co-exist, and cyanotoxin occurrence is highly variable and strongly influenced by the environmental conditions, the only way to confirm the presence and level of toxin is specific toxin analysis. Although (as suggested in the previous Section) health effects, especially the milder and self-limiting ones, may not be related to the known toxins.

A key issue in determining whether a waterbody is safe for recreational use is the sampling strategy. This is true for most parameters but, due to their (sometimes extreme) heterogeneous distribution in space and time, sampling cyanobacteria can be particularly challenging [47]. Scum forming

cyanobacteria, for example, can regulate the depth at which they accumulate and may be seen to appear (or disappear) at the surface within half an hour. Furthermore, when scums do form the wind will cause them to accumulate along shorelines or may cause them to disperse. Thus, for sampling it is important to decide whether the sample is to represent the overall cyanobacterial biomass (to provide an overview of cyanobacterial proliferation in the waterbody) or to determine a maximum concentration (e.g. where scums accumulate), or whether both are required.

D5.1 Cyanobacterial observations

There are a number of methods to establish the level of cyanobacteria in water from straight-forward visual examination (the presence of scum or a measure of turbidity) to the use of sophisticated remote sensing. In between there is microscopy, which can include identification, cell counting and measures of cyanobacterial biovolume. Also included in this section is the determination of phycocyanin (PC) – a cyanobacterial-specific pigment.

Satellite data

A relatively recent development has been the use of satellite remote sensing imagery to identify and track HABs (using, for example, the radiance of chlorophyll-a [Chl-a], preferably in conjunction with PC in cyanobacterial blooms). A system is under development in the USA which will allow access to information derived from satellite data analysis via a mobile app. The development version is presently showing cyanobacteria cell count data, but later versions are expected to include measure of Chl-a, turbidity and water temperature [(48)]. Similar developments are also occurring in Europe. There are, however, a number of obstacles to the widespread employment of remote sensing for water quality monitoring purposes, including the limited availably of satellite sensors for this purpose and because sensor resolution is limited detection is suited to large blooms in large water bodies [(12)]. In addition, there will be a low frequency of readings from non-stationary satellites (e.g. approximately once every 15 days) and weather limitations (a cloudy day will prevent readings being taken). Remote sensing cannot directly detect toxins so, in common with other non-toxin related methods, toxicity can only be inferred by establishing a relationship between toxin concentration (from sampling the water body) and the surrogate (e.g. phycocyanin) observations [(49)].

Microscopy: identification and cell counts/biovolume

Traditionally, light microscopy (used for species/genera identification and determination of cell counts) has been the most widespread method for monitoring cyanobacteria and the development of HABs. Rapid and simple methods can be used to establish the composition of a sample at a genera (rather than species) level and this can be sufficient for an initial assessment of the potential hazard [(41)]. In comparison many analytical methods, cell counting is considered to be time-consuming. There are European standard methods for enumeration of phytoplankton [(50)] and for the estimation of phytoplankton biovolume [(51)] and different European laboratories are accredited to perform these analyses. Although the WHO Guidelines [(16)] are based on suggested cyanobacterial cell counts, because cell size can vary considerably both within and between species, the determination of biovolume (rather than simple cell counts) is gaining favour and will be recommended in the upcoming edition of the WHO publication - Toxic Cyanobacteria in Water [(13)]. Biovolume requires the additional determination of the average cell volume (on the basis of measurements from, typically, 10-20 cells from each identified species; this is then multiplied by the cell number. The time needed for microscopy can be limited to a few minutes per sample if samples are only analysed qualitatively or semi-quantitatively and pigment concentrations are used as an

indicator of their quantity. Where pigment concentrations are used, a brief qualitative microscopic analysis is useful to assess which cyanobacterial species dominate in a given sample.

Phycocyanin

PC is an accessory pigment to Chl-a and is essentially specific to cyanobacteria, which can be measured in vitro (after pigment extraction from the cells) or in situ. It can be simply measured on site (using a hand-held fluorimeter) and has been shown (in combination with secchi depth) to be a good screening tool to estimate the probability of a beach exceeding acceptable MC concentrations at a number of eutrophic lakes in the USA [52]. In addition, there are also fluorometric probes that allow the selective excitation of other accessory pigments to allow differentiation between major taxonomic groups of phytoplankton organisms and allow quantification of phytoplankton biomass [53]. While studies have often shown good relationships between on-site fluorometric methods and laboratory measures, there are a number of factors that can limit the accuracies including total suspended matter, eukaryotic algal presence, variations in cyanobacteria community composition and also bloom conditions [54]. If a high level of precision is required, systematic study of the phytoplankton in the respective lake may be useful in order to develop comprehensive correction procedures for the full range of available probes [55]. However, for many surveillance purposes, inaccuracies due to other factors (see above) will far outweigh those due to quantification methods)

D5.2 Indicator/surrogate measurements

Chlorophyll-a

Chl-a measurement has been another mainstay of HAB monitoring, and an International Organization for Standardization (ISO) method is available [56] for laboratory analysis. It does not, however, discriminate cyanobacteria from algae, which can "*pose a serious limitation on data interpretation*" [57]. Although the combined analysis of Chl-a and PC (or of Chl-a and a quick microscopic check to determine whether cyanobacteria dominate and are likely to contribute the major share of Chl-a) can provide useful information on the proportion of cyanobacteria among other phytoplankton species.

Transparency

The transparency of the water at the bathing site can easily be measured using a Secchi disc. Where the transparency is low (less than 1 to 2 m), especially when accompanied by "*greenish to blueish discolouration, streaks or even scums high cyanobacterial densities are likely*" [44]. If the water is turbid (e.g. from clay), the colour is usually different (brownish for clay or blue to jade for limestone). Turbid water may enhance cyanobacterial dominance (if nutrient levels are sufficiently high) as, under low light conditions, the growth rates of some cyanobacterial species are higher than those of other phytoplankton.

Total phosphorus

This provides information on the potential for cyanobacteria proliferation; and it is total phosphorus that should be measured rather than dissolved phosphate (also known as orthophosphate). There is an ISO method available for this determination [58]. The likelihood of blooms has been found to increase at concentrations above 20 – 50 µg/l [59].

D5.3 Toxin analysis

There are a number of methods which can be used to detect and/or measure levels of a number of cyanotoxins in water [60] including:

- ELISA (enzyme-linked immunosorbent assay);
- PPIA (protein phosphatase inhibition assay), MC only;
- Reversed-phase high performance liquid chromatography (HPLC) methods, combined with mass spectrometry (MS), tandem mass spectrometry (MS/MS) or ultraviolet/photodiode array (UV/PDA) detection; and
- Polymerase chain reaction (PCR), quantitative PCR (qPCR) and microarrays/DNA chips.

These methods use different approaches [61], such as direct measurement of toxins (e.g. HPLC, LC/MS), measuring antibodies raised against the toxin (ELISA), toxicity of MC (PPIA) or estimating the potential for toxin production, based on determining the presence of toxin-producing genes (PCR, qPCR). Some methods (e.g. ELISA) are only semi-quantitative and do not distinguish between the different toxin variants, while a number of the methods are prone to matrix effects which affect the accuracy of the results. In order to compare toxin concentrations from different studies it is important to know the method of analysis, possible matrix effects, the performance of sample preparation techniques (e.g. recovery levels) and whether free, bound or total toxin form was measured [10].

Gaget [22] recently reviewed the pros and cons of a number of these methods for determining cyanobacterial toxins and toxicity (including ELISA, PPIA, PCR and chemical analysis) and concluded that there is no 'gold standard' technique, with each method having its own strengths and weaknesses. Method choice will depend on the sample type (e.g. drinking-water or recreational water) and also the choice of detection assay will depend on "*cost, practicality, reliability and comparability of results and essentially on the question to be answered*".

Some of the methods (e.g. immunochromatography, ELISA and PPIA) have been used to create field test kits for the detection of MC. While these have potential advantages, they are at a fairly early stage of development and are not without problems [12, 21]. Studies have shown that there can be difficulties with the visual interpretation of the results, it is important to understand the scope of the chosen kit (e.g. free [i.e. dissolved] toxin versus total toxin [i.e. including the cell-bound fraction]; qualitative versus semi-quantitative).

D6. Bathing water profile and potential beach classification

As outlined in D1, consideration of the proliferation potential of HAB organisms (cyanobacteria and marine phytoplankton) is a requirement under the bathing water profile. Where appropriate, Member States are then required to take adequate management action.

In 2010, a review of country-level practices for controlling the hazards from toxic cyanobacteria was conducted [62]. The approaches employed by a number of Member States were documented and these are summarised below (Table D3), along with data drawn from additional sources [15, 63].

Most of the countries listed in Table D3 have two or three action levels, with a typical management action at the highest level being to close or strongly advise against using the site. The specified cell counts (<20,000 – 100,000 cells/ml) and Chl-a levels (< 10 to >50 µg/l) are typically based on the

WHO guidelines [16]. The key transparency depth is 1m. Where countries use toxin measurements these are generally done at higher alert levels (i.e. not for routine monitoring purposes) and are to back up the decision-making process and/or to justify the thresholds for cyanobacterial biomass that trigger action; most countries consider MC concentrations of less than 20 µg/l acceptable. While the advantages of using parameters other than toxin concentrations are speed of decision-making, economic and allow for the potential impact caused by other known and unknown toxins, it has been noted that (in one study) the estimated risk based on using cyanobacterial abundance and chlorophyll metrics was higher than the estimates based on MC level [23]. Thus, a further role of toxin analysis can be de-warning.

Table D4: Cyanobacterial-related parameters used in European regulations/guidance

	Parameters									Diff. action levels
	TP	**CB cell count**	**CB BV**	**Cyano-Chl-a**	**Chl-a***	**Visual**	**Scums or foam**	**Transp**	**Toxin determination**	
DE	√		√	√	√	√	√	√	√ (for de-warning)	√
DK		√			√	√	√		+/-	√
ES	Parameters not specified									
FI						√	√	√		√
FR		√				√	√		√	√
HU		√			√				√	√
IT	√	√				√	√	√	√	√
NL				√	√	√	√			√
PO	Parameters not specified									
SO		√			√		√			√

DE: Germany; DK Denmark ES Spain FI: Finland FR: France HU: Hungary IT: Italy NL: Netherlands PO: Poland SO: Scotland
TP: total phosphorus CB: cyanobacteria BV: biovolume Cyano-Chl-a: cyanobacterial chlorophyll-a
* Some countries specify that for Chl-a (chlorophyll-a) measurements there should be a dominance of CB checked for by microscopy
Transp: transparency +/-: used in some regions

As noted [23], the threshold levels for cyanobacterial biomass are typically based on worst-case scenarios of toxin/biomass ratios (i.e. they are conservative). Where certainty about an action is required (e.g. keeping a site open, despite the presence of pronounced cyanobacterial biomass), toxin analysis can be worthwhile.

An alternative approach is used in Oregon (USA), where a toxin-based monitoring programme for recreational water sites is promoted. A number of benefits of such monitoring are suggested, including being about to "*communicate with the public about actual risks, as opposed to the potential risk represented by cell count data alone*" and reducing the risk of "*advisory fatigue*", where people stop responding to advisories because they are perceived as being so frequent that they no longer command attention [64].

D7. Conclusions

The stated purpose of the BWD is "*to preserve, protect and improve the quality of the environment and to protect public health*" (Article 1). The focus in this fact sheet and the WHO recommendations, however, is solely on health protection.

The current system for marine phytoplankton (i.e. consideration within the bathing water profile) should be retained.

Locations at risk of freshwater cyanobacterial blooms should be subject to a new classification/management system. This will be separate from the current FIO system (based on ENT and *E. coli*) as the sources are different, as are bloom behaviour and management responses. The new system should be based on the guidance levels, which are currently in preparation, suggested by the WHO [(13)] and should allow Member States to choose which parameters to monitor (e.g. biovolume, chlorophyll-a, phycocyanin, transparency and toxins). Work is currently underway to clarify the correspondence between these different parameters.

A clear on-site indication (informing the users) of cyanobacterial bloom risk should be given at a location which is identified as 'at risk' (e.g. “prone to harmful blooms”), and a standard symbol would be useful. In addition, there is a greater role for public information, allowing users to make an informed choice about recreational water activity.

The overall likelihood of cyanobacterial occurrence and proliferation can be predicted from information on water-body characteristics and history. Modelling cyanobacterial occurrence potentially has a valuable role to play in day-to-day management decisions. Available models and research results need to be collated and consolidated to provide guidance for Member States.

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E: Emerging/wider issues

The issues covered in this fact sheet were raised during the course of discussions with bathing water experts and stakeholders. Each area is briefly outlined and followed, where applicable, by WHO recommendations.

E1. Antimicrobial resistance (AMR)

The development of AMR is a natural phenomenon in microorganisms, which is accelerated by the selective pressure exerted by use and misuse of antimicrobial agents in people and animals and their environmental release [(1)]. There are a number of mechanisms by which microbes can develop resistance to antimicrobial agents [(2)], including:

- Inactivation or modification of the antimicrobial;
- Target site alteration, leading to a reduction in the binding capacity of the antimicrobial;
- Modification of metabolic pathways to circumvent the antimicrobial effect; and
- Reduced intracellular antimicrobial accumulation as a result of a decrease in permeability and/or increasing active efflux.

Some microbes are naturally resistant to certain groups of antimicrobial agents (as they lack the specific target of the agent), however, acquired resistance to antimicrobials can develop through the mutation of existing genes (vertical evolution) or by the acquisition of new genes from other strains or species (horizontal gene transfer). There are a number of bacterial mobile genetic elements (including plasmids and transposons) which facilitate horizontal gene transfer [(2)].

According to Wellington [(3)], the reservoir of resistance genes in the environment is due to a mix of naturally occurring resistance, resistance genes present in animal and human waste and resistance developed in response to the presence of antimicrobial agents in the environment released from pharmaceutical manufacturing or, indirectly, after passing through the human or animal body because of their selective effect.

AMR can be detected in growth inhibition assays and, while research on antimicrobial resistance has focused mainly on the clinical setting [(2)], a brief examination of the literature reveals a number of studies conducted in European recreational waters, including Greece, Spain, Poland, Netherlands, Germany, England & Wales, Croatia and Norway [(4-12)]. These have targeted various bacteria (including *Enterobacteriaceae*, enterococci, *E. coli*, heterotrophic bacteria, *Vibrio* spp. and vibrio-like organisms) and considered a range of different antibiotics. The findings varied according to location, bacteria targeted and antibiotics studied, but some level of resistance was found in each study. In Greece [(4)], for example, over 87% of enterococci exhibited acquired resistance to one or more antibiotics, with the most frequent pattern seen being resistance to both erythromycin and rifampicin. In Croatia, resistance was seen in *Enterobacteriaceae* isolated from 3 public beaches to all 13 of the antibiotics tested, with the highest prevalence of resistance (92%) seen against ampicillin [(11)].

The presence of antimicrobial resistant strains in such environments has led to speculation that beaches and recreational waters may be a reservoir for possible transmission of antimicrobial resistant bacteria to water users and beach visitors [(10, 13-15)]. This has received support from recent

epidemiological research conducted in the UK [16], in which a cross-sectional study was conducted to assess if there was an association between surfing and gut colonisation by *E. coli* with a specified plasmid-borne antimicrobial resistant gene (bla_{CTX-M}). Gut colonisation (determined by rectal swab) by resistant *E. coli* was determined in 143 surfers and 130 non-surfers. Surfers were approximately three times more likely than non-surfers (13 versus 4) to be colonised by cefotaxime-resistant *E. coli* (risk ratio 2.95; 95% CI 1.05-8.32) and 9 surfers were found to be carriers of bacteria with one specific type of resistance mechanism against cefotaxime, that is bla_{CTX-M}-bearing *E. coli*, compared to 2 non-surfers (risk ratio 4.09; 95% CI 1.02-16.4). However, surfers may be exposed to other types of water than designated bathing sites, and thus these findings may not be relevant for evaluating the possible risk associated with bathing water. Further work is required to establish the acquisition of resistant microbes from coastal waters and other natural environments which have been identified as AMR reservoirs.

E1.1 Conclusions

Recreational water exposure is unlikely to be a major route of transmission of antimicrobial resistant microorganisms in the European Union compared with other routes. Based on evaluation of the currently weak evidence regarding transmission of resistant bacteria through bathing water combined with the need to develop surveillance methods for application in recreational waters, it is not recommended that such surveillance be initiated at this moment. This should, however, be re-assessed in the event of potential future revisions to the BWD, in coordination with consideration of options for harmonized monitoring of AMR in the environment under 'European One Health Action Plan against Antimicrobial Resistance' [17].

Source control may be a more fruitful means of reducing environmental exposure to resistant microorganisms and consideration should be given to liaison with the European Medicines Agency (to identify European regions where it may be feasible to encourage a reduction in use) and the potential inclusion of AMR in the Urban Waste Water Treatment Directive (to reduce environmental levels of both antimicrobial residues and resistant microorganisms).

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E2. Microplastics

The mass production of plastics began in the 1940s and global annual plastic production has shown continuous growth for more than 50 years and has increased from less than 2 million tonnes in 1950 to approximately 335 million tonnes in 2016 [18].

The problem of plastic waste, its indiscriminate disposal and the impact of large plastic debris on the marine environment has been recognised for some time, and it has been estimated that up to 10% of plastic produced will enter the sea [19]. In addition to the aesthetic issues, environmental impacts include [20]:

- the injury and death of birds, mammals and fish (etc.) as a result of plastic ingestion, entanglement or suffocation;
- transport of non-native species to new habitats (on floating plastic debris); and
- the smothering of the seabed.

In the 1970s, the presence of small plastic fragments in the open ocean was noted, and these are now considered to be a pollutant in their own right [20, 21]. Microplastics are a "*heterogeneous mixture of particles ranging in size from a few microns to several mm in diameter; including particles of various shapes from completely spherical to elongated fibres*" [22]. They are now generally accepted to be <5 mm in size [23]. They have been described as "*widespread and ubiquitous*" [20] and have been reported in lakes as well as in marine waters [22] and Arctic Sea ice [24].

Microplastics can be divided into primary and secondary microplastics. Primary microplastics are those which are manufactured to be <5mm and include plastics used in facial cleansers and cosmetics and also those used as air-blasting media [20]. Secondary microplastics are derived from the breakdown of larger plastic debris.

Information on the impact of microplastics on marine organisms [25] and potential impacts on humans (via the food chain) is currently lacking [26, 27]. Although a number of issues have been highlighted including the negative impacts from the actual ingestion (such as pseudo-satiation and reduced food intake) and also possible toxic effects following ingestion [20, 28]. The toxic effects could derive from inherent contaminants (e.g. phthalate plasticizers) or from persistent organic pollutants (POPs) adsorbed to the plastic which may become bioavailable following ingestion. POPs and metals

have been shown to sorb to plastics and can become orders of magnitude more concentrated on the surface of the plastic than in the surrounding water [20]. It has, however, been pointed out that "*microplastic ingestion may either clean or contaminate the organism, depending on the chemical fugacity gradient between ingested plastic and the organism tissue*" [29].

The research gap on sources, pathways and impacts of microplastics is rapidly being addressed, as illustrated by two recent reviews [30, 31] commissioned by the European Commission (one on primary- and one on secondary-microplastics). The Commission has asked the European Chemicals Agency (ECHA) to prepare a dossier for REACH restriction[8] and, in March 2018, the ECHA launched a call for information.

While there is currently no conclusive evidence pointing to human health impacts, research is ongoing and it can be expected that information may be available to allow the possible impacts on bathing water and the BWD to be assessed. It should be noted, however, that microplastics fall fully within the scope of the 2008 Marine Strategy Framework Directive (MSFD), which aims to establish good environmental status of European seas by 2020. Good environmental status is defined using 11 qualitative descriptors outlined in Annex I of the MSFD. Descriptor 10 ("*Properties and quantities of marine litter do not cause harm to the coastal and marine environment*") is relevant to microplastics [32].

E2.1 Conclusions

The issue of microplastics falls within the scope of the Marine Strategy Framework Directive, notably through the implementation of Commission Decision 2017/848/EU that lists micro-litter as one of the criteria elements which EU Member States have to consider in their marine strategies. Ongoing research will help us understand in the short to medium term whether microplastics are also relevant for inclusion within the BWD.

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E3. Other infectious agents

Although the BWD focuses on the protection of bathers from microbial infection, the microorganisms used (enterococci and *E. coli*) are indicators of faecal pollution and do not capture the possible impact of naturally occurring (autochthonous) microorganisms. Member States have highlighted two issues of concern, namely:

- Swimmer's itch (cercarial dermatitis); and
- *Vibrio* spp.

E3.1 Swimmer's itch

Swimmer's itch is a, generally, harmless but unpleasant skin reaction caused by exposure to schistosomes. Cases have been reported in a number of European countries including: Austria, Czech Republic, France, Germany, Iceland, Italy, Netherlands, Norway, Spain, and UK. The most common causative agents are avian schistosomes (*Trichobilharzia* spp.), which have a complex life cycle involving freshwater snails and waterfowl. Humans are an accidental host and the cercariae (mobile larval stage released from waterfowl) generally die shortly after skin penetration, with the inflammatory skin reaction being caused by the host's immune response [(33)].

Eutrophication has been associated with increasing cases of swimmer's itch. The resulting increase in biomass caused by the eutrophication has been linked with ideal conditions for abundant snail populations which, in turn, can result in greater colonization of freshwaters by waterfowl. In addition, climate change is also likely to be an important driver as it has been shown that trematodes are very sensitive to temperature changes and both cercarial production and emission rates have been found to be temperature dependent [(33)]. In addition to environmental factors, personal swimming behaviour is also likely to have an effect on the likelihood and severity of symptoms [(34)].

E3.2 Vibrio

Vibrio infection is an emerging disease in Europe [(35)] which has been related to an increase in surface sea temperature [(36)].

Pathogenic vibrios produce a range of infections. Species which have been identified in European waters [(37-38)] include:

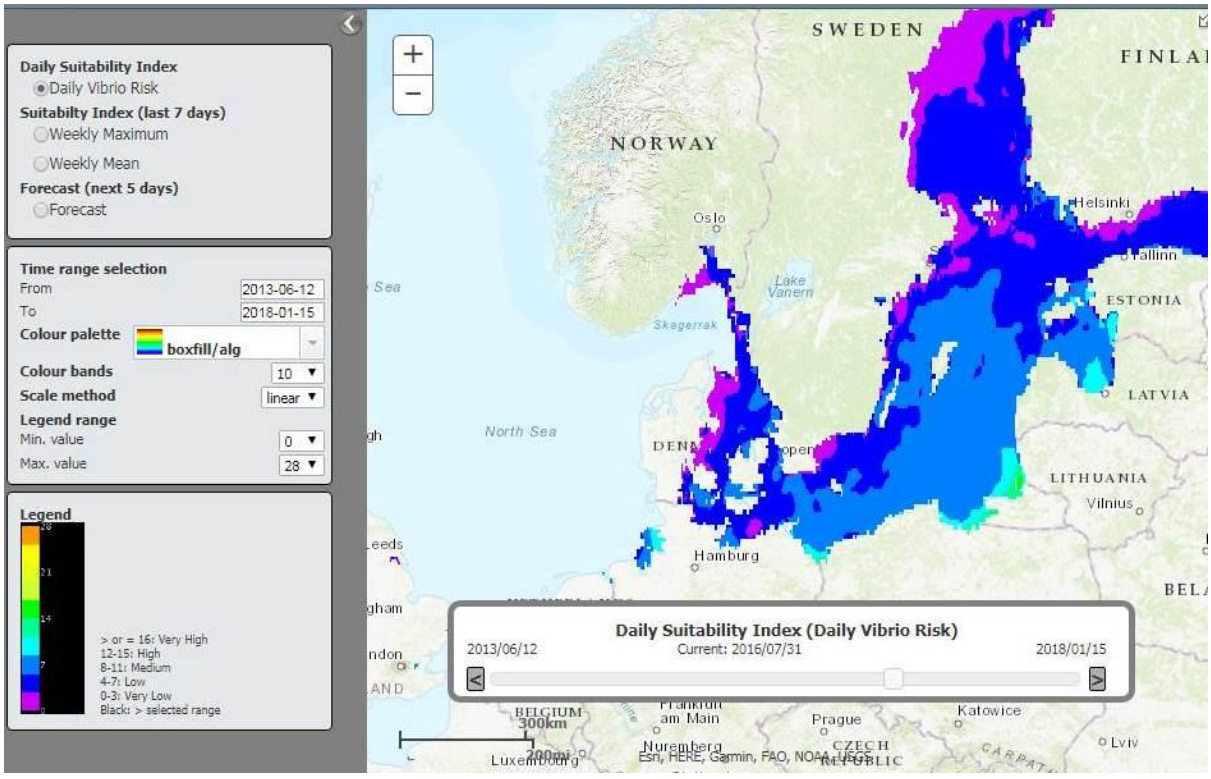
- *V. alginolyticus*;
- *V. cholerae* non-O1-non-O139;
- *V. parahaemolyticus*;
- *V. vulnificus*.

While *V. parahaemolyticus* and *V. cholerae* non-O1-non-O139 are principally associated with food poisoning following consumption of raw or insufficiently cooked seafood they can also, along with *V. alginolyticus* and *V. vulnificus*, cause wound infections following exposure to contaminated water or cleaning/handling seafood [39-44]. Cases of vibrio wound infections and/or septicaemia have been reported from a number of European countries including Germany, Austria, Sweden, Finland, Denmark, Poland, the Channel Islands, the Netherlands and Spain [38, 45-46].

It is likely that the recent increase in prevalence of vibrios in Europe is related to climate change. Vibrios preferentially grow in warm (>15°C) saline water environments and the sea surface temperature in coastal European waters has increased, in the last few decades, between four and seven times faster than in global oceans [36]. Samples collected as part of a continuous plankton recorder survey have shown that vibrios have increased in prevalence in the North Sea over that last 40 years and that the increase is correlated with the sea surface temperature [36].

A tool has been developed by the European Centre for Disease Prevention and Control (ECDC) to assess the environmental suitability of coastal waters for vibrio blooms. It is a real time model that uses daily updated remote sensing data (including sea surface temperature and salinity) to determine the likely hazard posed by vibrios (https://e3geoportal.ecdc.europa.eu/SitePages/Vibrio%20Map%20Viewer.aspx).

The figure below (E1) shows the model results for 31st of July 2016.



FigureE1: Daily Vibrio risk for 31/7/16 from the E3 Geoportal (ECDC)

E3.3 Conclusions

At locations where swimmer's itch is known to occur, this should be included in the bathing water profile and information provided to members of the public.

In addition to *Vibrio* spp., there are a number of other microorganisms that can cause wound infection following exposure to recreational water, such as *Aeromonas* spp. (principally fresh water).While *Leptospira* spp. (freshwater) can gain access to the body via wounds exposed to contaminated recreational water. It is suggested that where cases of such infection types have resulted from a recreational water exposure that this information be covered in the bathing water profile and advice given on bather hygiene measures to minimise risk and actions to take if a wound is sustained while bathing.

E3.4 References

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E4. Bather definition

The limited scope of 'bathing' (limited in the BWD to activities associated with swimming and bathing) was raised during one of the break-out sessions at the November Stakeholder Consultation meeting.

Although the BWD does not provide a definition of bathing, Article 1(3) of the BWD limits its scope to "*any element of surface water where the competent authority expects a large number of people to bathe*". It further defines (in Art. 2(4)) a large number as a number "*that a competent authority considers to be large having regard, in particular, to past trends or to any infrastructure or facilities provided, or other measures taken, to promote bathing*".

The issue of other, non-bathing activities, was recognised in the 2002 proposal for the revision of the 1976 BWD [(47)], where the Commission stated that new patterns of recreational water use presented significant challenges as they were practiced at significant distances from the shore. It was also noted that the practitioners of activities other than bathing or swimming would very often use

places considered unsuitable for swimming. Moreover, recreational water-sports (thanks to development of new materials) were undertaken outside the bathing season and due to the nature of the activities may require the division of a bathing area into different zones. On this basis, the Commission has taken the view that it would not be appropriate to include additional recreational uses of water in the definition of bathing waters as this would oblige Member States to significantly increase the extent, both physically and temporally, of water quality protection, monitoring and management obligations.

E4.1 Conclusions

It is acknowledged that a wide variety of recreational water activities may take place at bathing water locations but to specifically take account of these activities would potentially require different (and additional) sampling locations, an extended sampling period (as some activities take place outside of the traditional bathing season) and a possible zoning of the bathing area.

Although a recommendation on the scope of bathing was not requested by the EC, it is suggested that the widening of scope of the BWD, currently restricted to bathers, could be re-considered in future if the non-bathing use of bathing sites continues to increase.

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Appendices

1. Membership of the WHO water quality technical advisory group

2. Meeting details

- 24-25th January 2018, Geneva, WHO expert group meeting on recreational water
- 24th November 2017, Brussels, Stakeholder's meeting on WHO recommendations relevant to the parameters for bathing water quality in the BWD
- 5th October 2017, Brussels, Meeting of the EC informal experts group on the implementation of Directive 2006/7/EC (Bathing Water Directive)
- 22nd September 2017, Ispra, European Microbiology Expert Group

Appendix 1. Membership of the WHO water quality technical advisory group

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Appendix 2. Meetings

Contributions from a range of experts, stakeholders and Member States were received during the course of the review process, principally through feedback on draft documents and through a series of meetings. The meetings are summarised in the following sections (starting with the most recent).

2.1 WHO expert group meeting on recreational water (24-25/01/18)

2.1.1 Introduction

This meeting was the culmination of the review process in which the results of the literature review and contributions received from other expert groups, stakeholders and EC Member States were distilled into 'aspects for consideration' (as shown on the agenda). These aspects were discussed at the meeting and lead to a series of scientific recommendations relevant to the BWD, which are detailed under the relevant fact sheet in the main body of the report.

2.1.2 Agenda

World Health Organization

AGENDA

Expert Group meeting on Recreational Water Quality

24 to 25 January 2018, Geneva, Switzerland

Day 1 – 24th Jan 2018

8:45 - 9:00 arrivals

Session1: 9:00 - 10:30 Introduction **Chair: Kate Medlicott**

- Welcome remarks
  - WHO –Kate Medlicott
  - EC Maja Feder
- Meeting Objectives and declaration of interest
- Introduction of participants
- Context and setting the scene – Lorna Fewtrell
- Discussion

Session 2: 11:00 - 12:30 Current parameters **Chair: David Cunliffe**

- Review of outstanding items in the Faecal Indicator Organisms factsheet and agreement on recommendations for EC BWD.

Aspects for consideration

- The current use of different percentile values for the different classification levels (i.e. 95%ile for 'excellent' & 'good' and 90%ile for 'sufficient') is confusing and difficult to explain
- Results from many sites do not exhibit the assumed $\log_{10}$-normality
- Censored data (i.e. < and > results) may have an impact on classification (should dilution policy be specified?)
- Sample numbers (minimum is 16 - misclassification)
- Spatial & temporal variability in water quality (the emerging issue in the USA and EU)
- Provision of additional guidance on day-to-day management (as distinct from long-term classification), e.g. some countries already have threshold values (above which they will advise against bathing).
- Europe is not ready to use qPCR methods for regulatory purposes (there is, presently, no driver and no Europe-based

epidemiology) – an area for future consideration?
- The current classification is based on 4-years monitoring data (the key is not years but the 'n' value for %ile based standards). In some cases, where no major changes have occurred in the catchment, this could be extended (more likely to be applicable to coastal sites)

Session 3: 13:30 - 15:00 Potential parameters **Chair: Tim Wade**

- Review of outstanding items in the Harmful Algal Blooms factsheet and agreement on recommendations for EC BWD.

Aspects for consideration
- There is support for inclusion and a flexible/pragmatic (pick-list) approach: What parameters and how to ensure equivalency between the different choices?
- Where do the WHO toxin guidelines (drinking-water) fit in?
- Part of the overall classification/separate classification?
- Role for public education
- Role for bloom/toxin prediction modelling
- Climate change

- Review of outstanding items in the Viruses factsheet and agreement on recommendations for EC BWD.

Aspects for consideration
- Assumption: It is not currently suggested that a viral indicator be included in recreational water guidelines or regulations, although these should be considered for future revisions
- Virus use could be suggested as part of MST & QMRA
- Research needs: Coliphage epidemiology? Viral parameter choice (e.g. consistent shedding patterns)? Others
- …

Session 4: 15:30 - 17:00 Tools & discounting **Chair: Calum McPhail**

- Review of outstanding items relating to tools, modelling and discounting and agreement on recommendations

Aspects for consideration
- Validation of models (% of misclassification, explained variance)
- Requirements for a clear and transparent (justifiable & auditable) approach at country level if modelling is used (e.g. re choice of risk predictors and model type)
- BWD discounting level is currently 15%
- Specific (optional) tools, such as MST & QMRA, could be incorporated into the beach profiling process

Day 2 – 25th Jan 2018

Session 1: 8:30-9:30 Conclusions for BWD and emerging issues for wider consideration by EC

Chair: Teresa Lettieri

- Recap of recommendations from Day 1 (Lorna)
- Wider issues to be covered in the chapeau of final report to EC (including antimicrobial resistance, 'bather' definition, *Vibrio*, microplastics)

2.2 Stakeholder's meeting on WHO recommendations relevant to the parameters for bathing water quality in the BWD (24/11/17)

2.2.1 Introduction

This meeting was designed to allow European stakeholders with an interest in bathing water quality to contribute to the review process. It combined presentations[9] with interactive discussion sessions. Participants were provided with the draft fact sheets in advance of the meeting. Participants were invited by Matjaž Malgaj, Head of Unit C.2 - Marine Environment and Water Industry, Directorate General for Environment.

2.2.2 Agenda

Ref. Ares(2017)5188268 - 24/10/2017

Stakeholder consultation meeting on WHO recommendations relevant to the parameters for bathing water quality in the Bathing Water Directive 2006/7/EC

Albert Borschette Congress Center (CCAB) Rue Froissart 36, 1040 Etterbeek/Brussels Room 1B

November 24th 2017 9am-4pm

The presentations will be based on four draft factsheets (covering the current parameters and possible viral and harmful algal bloom parameters) which will be available and distributed in advance of the meeting.

This will be an interactive meeting with contributions and discussions actively sought. Please be prepared to get involved.

Timings	Activities	Presenters/Facilitators
9.00	Welcome, housekeeping and how the day will work	Matjaž Malgaj (EC/DG ENV)
	Update on the on-going grant agreement with the WHO	Maja Feder (EC/DG ENV)
	World Health Organization involvement	Kate Medlicott (WHO)
	Current and possible parameters: what the literature tells us	Lorna Fewtrell *(WHO consultant)*
	Questions/Discussion	Lorna Fewtrell *(WHO consultant)*
10.15	Morning break	
10.30	Methods for water quality analysis	Teresa Lettieri (JRC)

[9] Available in the meeting folder on CIRCABC

	The regulatory development of prediction and discounting (modelling)	David Kay (WHO expert group member)
	Group discussions (3 groups)	Lorna Fewtrell, David Kay, Kate Medlicott, Teresa Lettieri, Maja Feder
12.00	Lunch	
13.00	Groups report back to plenary, possible further discussion	
	Classification of bathing waters. An analysis of water quality data and the results of a recent Member State questionnaire	Lidija Globevnik (EEA - ETC/ICM Waters)
	General discussion/feedback on factsheets	Lorna Fewtrell (WHO consultant)
14.45	Afternoon break	
15.15	Summing up	David Kay (WHO expert group member)
16.00	Closure of the meeting	

2.2.3 Group discussion questions

Each person had the opportunity to contribute to two of the three areas.

1. FIO parameters (enterococci, *E. coli*, viral parameter)

What is the best way to protect human health?

- A long term classification
- Information on which to base day-to-day decisions (role for prediction?)
- A combination

Thoughts on whether *E. coli* should continue to be measured in marine waters

Thoughts on whether enterococci and *E. coli* should be measured at each site

Thoughts on whether a higher concentration of enterococci is acceptable at fresh water sites compared to coastal sites

Would a change be useful /beneficial (or is no change better)?

Is additional work required before changes can be implemented (e.g. viral methodology/epidemiology)?

Thoughts on the best way to deal with the inclusion of specific methods within the BWD

Thoughts on culture versus rapid methods

2. Methods/HABs

Is there a need for specific guidance and consistent levels for HAB-related parameters?

Should HABs be a formal part of the classification or is information on which to base day-to-day decisions more appropriate?

Is a flexible/pragmatic approach (allowing countries to choose the parameters for measurement) desirable?

Is there a role for toxin measurement (e.g. as part of the decision-making process or as a 'de-warning' option)?

How should a country deal with lots of problem sites?

Is there a role for public education?

- If so, what form?

Is there a role for modelling?

3. **Prediction and discounting**

Thoughts on regulatory sample numbers

Is compliance data suitable for model building or does within-day variability need to be accounted for?

Should prediction and discounting only follow a sanitary survey suggesting 'qualitatively' no-human faecal pollution?

Should the discounted samples be limited to 15% at EU beaches with no sanitary survey filter?

Could greater use be made of discounting (where people are discouraged from entering the water)?

Could a similar discounting approach be used for HABs?

2.2.4 Meeting feedback

The meeting feedback points have been revisited in light of the WHO expert group meeting and the fact sheet finalisation process and an update column has been added.

REC - recommendation

Key stakeholder feedback points:	**Follow up**	**Update Jan-18**
Current situation		

• Although the BWD is the only major set of regulations that requires measurement of *E. coli* at marine sites and the measurement of both *E. coli* and enterococci, it was felt that there was a role of *E. coli*: ▪ people are familiar with it and it is similar to drinking water; ▪ at some sites it drives compliance with the BWD; ▪ it may well have a role to play in new issues, such as anti-microbial resistance; ▪ its measurement (using culture methods) does not add greatly to costs; ▪ continued analysis provides trend information.	A comment will be added to the Fact Sheet to this effect	Included
• The use of a 95%ile value for 'excellent' and 'good' and a 90%ile value for 'sufficient' was seen as confusing, and there was support for a 95%ile value to be used across all of the classifications.	Discussion with WQTAG	***REC***
• The bathing water profile was seen to provide useful information, which some participants felt should be more public.	No action – already in Fact Sheet	No action
• There was support for the position not to recommend inclusion of a viral parameter in the current BWD review.	No action required	***REC***
• HABs are considered as part of the bathing water profile. There was support for additional classification guidance within the BWD and a set of parameters (with associated levels, providing a comparable degree of protection) from which countries could choose for monitoring purposes. A parallel (non-legally binding) classification system – with additional or modified public information logos was seen as useful.	Discussion with WQTAG	***REC***
<u>Water quality analysis</u> • There is a requirement for an *E. coli* membrane filtration ISO method that is suitable for bathing waters. EC should raise the issues with ISO so that a method may be available in time for the 2020 revision. ISO 9308-2:2012, which was not available when the BWD was published and is in use in a number of MS should be included in the revision.	EC to discuss with ISO	***REC***
• It was felt that qPCR should be treated with caution (research rather than regulatory use), given the difference in results seen between culture and qPCR methods, and also, the difference in attenuation of parameters measured by qPCR and culture methods seen for disinfected sewage.	Discussion with WQTAG	***REC***
• The problem resulting from censored data (< or > data) and how this could impact on beach classification was raised. It was suggested that there could be a limit on the allowable number of censored values and that, maybe, additional advice on laboratory dilution practice would be useful.	Discuss with WHO expert group	***REC***
• Flexible parameter choice for HABs monitoring seen as useful, with approximate consistency of degree of protection between different measures.	This is currently being worked on (TCiW)	***REC***

• Given the number of different HABs toxins and the potential time taken for analysis, it was suggested that their analysis was most useful for 'de-warning' – i.e. potentially over-riding the action level provided by other measures (such as chlorophyll-a)	Discussion with WQTAG	Discussed, no specific REC
• Use of the minimum number of samples for classification (16) is not scientifically defensible and will result in significant misclassification. It was felt to be in Member States interests to increase the number of samples. A minimum of 80 to 100 is appropriate.	Discussion with WQTAG	***REC***
Prediction & discounting • Not many countries currently use prediction modelling, although it was seen as valuable in terms of providing day-to-day management information and informing bathers of on-the-day risks. Prediction models require validation, which could focus on the percentage of misclassification and the model explained variance.	Discussion with WQTAG	***REC***
• Because of wide differences between European sites, the risk predictors (e.g. river flow, presence of combined sewer overflows, microbial source tracking) and specific choice of model type (e.g. linear regression, decision trees; hydrodynamic process-based) is likely to be site specific. Thus, model, calibration, additional data collection and outcome indicators (including 95%ile value, threshold value, outcome based on health risk) should be done at country-level. The chosen approach and data choices must be clearly explained, justifiable and auditable.	Discussion with WQTAG	***REC***
• Prediction models could be used instead of a check sample to allow a return to use. This was felt to require further consideration and validation.	Research need	***REC***
• Guidance on the number of data points required for model building (especially when applied to marginal sites that fall in and out of compliance) – however, 16 samples (the BWD minimum over a 4 year compliance period) is insufficient.	Research need	***REC***
• Whilst the presently allowed 15% discounting level was felt to be practical, some felt that it was restrictive and should be higher, although the potential for public confusion was highlighted. WHO suggests where discounting is used that the classification is modified (e.g. Very good [but unsuitable for several days after rain]).	Discussion with WQTAG	***REC***
• Where discounting is used, it was suggested that countries need to define 'abnormal' conditions in advance.	Discussion with WQTAG	No action
• Prediction potentially has a role for HABs, but additional research is likely to be required before widespread acceptability.	Research need	***REC***
Classification		
• The current overall classification structure was, generally, supported; with the different levels being seen as important for informing investments and driving improvement. The 'Sufficient' classification has a valuable role to play and should not be phased out, although changing to a 95%ile	Discussion with WQTAG	***REC***

value (rather than the current 90%ile) was seen to improve coherence and consistency.		
• Additional guidance on day-to-day management (as distinct from long-term classification) was seen as useful – especially to provide the public information on the likely risk on a bathing day. This could be provided by using prediction modelling, or some countries already have threshold values (above which they will advise against bathing); and an optional EU-wide (or regionally-based) threshold level was suggested.	Discussion with WQTAG	***REC***
• It was suggested that the 4-year period could be increased to include more samples (provided no major changes have taken place) to change the quality of the classification.	Discussion with WQTAG	***REC***
• A flexible approach to parameter choice for HABs was preferred by stakeholders.	Discussion with WQTAG	***REC***
<u>General feedback</u> • Information on emerging issues, tools and techniques which are not ready for regulatory consideration in the BWD should be included. Possible areas for consideration include anti-microbial resistance, micro-plastics, microbial source tracking and other infectious agents (e.g. *Vibrio* spp.). These could be considered and/or provide links with other Directives (such as source control issues which could be addressed in the Urban Waste Water Treatment Directive	Research need Discussion with WHO expert group	***Additional Fact sheet (E)***
• Given the wide expansion of water-based recreational activities, 'bather' may be too restrictive, although there will be implications in terms of sampling locations, length of the season, additional sites and the management of wastewater treatment technologies outside of the traditional bathing season.	Definition of 'bather' to be taken up by the EC in the other project and process to complete BWD revision. It should be noted, however, that additional research may be required	***Covered in Fact Sheet E***
• Some areas may need specific guidance: ▪ The Baltic Sea, for example, is an area of low salinity and, in many ways, it may be more similar to a lake/freshwater site, than a coastal or transitional water. ▪ Some countries have specific recreational-water infection issues – such as Vibrio spp. and cercarial dermatitis (swimmer's itch). Guidance on country-level risk assessment may be appropriate.	Noted – no action needed (see above).	***Covered in Fact Sheet E***

Abbreviations

%ile percentile
< less than

>	greater than
BWD	Bathing Water Directive
E. coli	*Escherichia coli*
EU	European Union
FIO	faecal indicator organism
FS	fact sheet
HABs	harmful algal blooms
ISO	International Organization for Standardization
qPCR	quantitative polymerase chain reaction
TCiW	Toxic cyanobacteria in water – WHO document, 2nd edition currently being written
WHO	World Health Organization
WQTAG	water quality technical advisory group (WHO expert group)

2.3 Meeting of the EC informal expert group on the implementation of Directive 2006/7/EC (5/10/17)

2.3.1 Introduction

During this meeting a presentation[10] was given on the progress of the review process to date and an outline given of future steps. During the initial literature review phase, it was identified that little information would be captured on in-country experiences and possible concerns relating to bathing water quality thus, in addition, a questionnaire (reproduced below) was circulated to Member States in advance of the meeting.

2.3.2 Member State questionnaire

The questionnaire was discussed at the meeting. A number of Member States felt that in order to provide an appropriate level of feedback the completion date should be extended from 10/1/17 to 30/10/17. A total of 21 member States responded to the questionnaire by the end of October. The questions highlighted in blue were discussed (briefly) during the meeting. The questionnaire feedback was used to inform subsequent discussions.

Bathing Water Directive review questionnaire

Background

Article 14, paragraph 3 of the Bathing Water Directive (2006/7/EC) – BWD states that "*the Commission shall, no later than 2020, review this Directive with particular regard to the parameters for bathing water quality, including whether it would be appropriate to phase out the 'sufficient' classification or modify the applicable standards* …" As part of this review process a literature review has been conducted of the recent relevant bathing water literature, part of which will be presented at the meeting. While a literature review can provide information on the latest research, it does not capture in-country experiences related to the application of the BWD.

Instructions

Please answer the following questions (as far as possible), for the situation in your country. The questions highlighted in blue will (time-permitting) be discussed at the meeting. Please fill in the form in English (creating as much space as required), ensuring that your country is clearly stated at the top.

Ideally, please provide feedback, if possible in advance of the meeting or by Tuesday October 10th directly to Lorna (lorna@creh.demon.co.uk) with ENV-Bathing-Water@ec.europa.eu in copy.

COUNTRY: ……

Questions

Parameters

1. What, typically, drives compliance (i.e. enterococci or *E. coli*) at your:
   marine bathing sites?
   fresh water bathing sites?

[10] Available in the meeting folder on CIRCABC

……

2. What do you think the likely impact would be on the classification of your freshwater sites if the WHO (2003) coastal enterococci concentration values were adopted?

……

3. Is there any pressure in your country to amend the current parameters (e.g. introduce a viral indicator or formal cyanobacterial monitoring)? If so, from which agencies/organisations?

……

4. Do any of the bathing water profiles in your country suggest that cyanobacteria/harmful algal blooms may be a problem at:
   marine bathing sites?
   fresh water bathing sites?
   If yes, how do you monitor? Do you measure toxin concentrations?

……

Methods

5. What, if any, problems have you had with the ISO methods specified in the BWD?

……

6. Which method do you currently use for analysis:
   Enterococci?
   E. coli?

……

Bathing water classification

7. The BWD currently uses percentile evaluation to determine regulatory compliance (based on the assumption that the data are a $\log_{10}$ normal distribution). Many sites, however, have water quality data which are not $\log_{10}$-normally distributed. How would using the Hazen method (a non-parametric ranking method) impact on results for your bathing waters?

……

8. What is your opinion on the validity of using 95-percentile values for 'excellent' and 'good' and 90-percentile for 'sufficient'?

……

9. Do you feel that percentile values are the most appropriate regulatory limit (compared with threshold values or geometric mean values)?

……

10. The current BWD Enterococci standards for coastal waters roughly translate into the following probabilities of gastrointestinal illness in bathers:
    Excellent: 0 – 3%
    Good: 3 – 5%
    Sufficient: 5 – 8.4%
    Do you feel these levels are appropriate in your country? If not, what changes would be necessary?

……

11. What impact would removing the 'sufficient' classification have on bathing water sites in your country (e.g. perceived impact on health of bathers, investment of extra resources to ensure beaches could comply with 'good', de-designation of certain beaches)?

……

Bathing water profile

12. Approximately what proportion of bathing sites in your country would be classified as mainly impacted by:
    point source pollution?
    non-point source pollution?

……

13. The WHO Guidelines classification is made up of two components: the sanitary survey (which assesses the susceptibility of the bathing water to pollution from faecal pollution [akin to the bathing water profile], with a specific emphasis on pollution from human faecal sources) and the microbial water quality. Together, these aspects allow the site to be graded 'very good', 'good', 'fair' or 'poor'. Do you think integration of the bathing water profile into the bathing water quality classification (as per the WHO and its sanitary survey) would be useful?

……

14. If the data from the bathing water profile were incorporated into the classification, would you support an increased allowance for discounting (i.e. over the BWD value of 15%) where pollution is from non-human sources?

……

15. Do you currently use faecal source tracking (microbial source tracking) methods as part of the bathing water profile? If so, what methods are applied and how widespread is the use?

……

Public information

16. How widespread in your country is the use of real-time predictive modelling to inform the public of times to avoid entering the water with associated public information via signage, social media, text messaging or the internet?

……

17. Is there any interest/pressure in your country to provide on-the-day information to the public from a single morning sample analysed using rapid methods such as qPCR?

……

General

18. Do you have any other comments about the parameters/ values/classification system in the current BWD (not captured by the questions above)?

……

2.4 European Microbiology Expert Group (22/9/17)

The background paper on microbial indicators (enterococci, *E. coli* and possible viral indicators) was circulated to members of the EU Bathing Water Directive Expert Group and European Microbiology Expert Group (EMEG) and EC Joint Research Centre (JRC). Feedback is summarized below.

Key feedback points:	**Follow up**
General feedback • EMEG reviewer wanted it noted that WHO Guidelines also include information on beach sand quality	Noted in additional text although WHO GL sand is not included in the classification system
Faecal Indicator organisms	
• EMEG noted the need to cite literature describing environmental sources of ENT. Possibility to evaluate *E. coli*/ENT ratio could be added.	Note and include in fact sheet
• EMEG pointed out that in shallow brackish water ENT only may lead to overestimation of health risks	Noted
• EMEG requested that effects of disinfected waste water effluent are further discussed reflected – specifically how disinfection affect the ratio between FIO and more resistant pathogens and	Background document amended

there potential for underestimation of an infection risk in bathing sites receiving disinfected effluents.	
• FR discussed pros and cons of ENT and *E.coli* in fresh and marine water.	Noted
Viruses	
• EMEG point to additional studies: ○ *McBride et al*. Discharge-based QMRA for estimation of public health risks ○ *Vergara et al.* Risk assessment of noroviruses and human adenoviruses in recreational surface waters ○ *Hokajärvi et al*., 2013. Occurrence of thermotolerant Campylobacter spp. and adenoviruses in Finnish bathing waters and purified sewage effluents.	Studies included in review and background document amended
• EMEG noted the application of coliphages should be further evaluated before a decision is taken. • FR cautions against the reliability and cost of such testing	Key aspect for consideration at WHO WQTAG
• EMEG reviewers had conflicting views on whether AdV is probably the most promising potential viral indicator.	Key aspect for consideration at WHO WQTAG
Cyanobacteria	
• EMEG /JRC noted cyanobacteria is important indicator for consideration • FR noted the increased complexity and diversity of species and those which are pathogenic, those which produce toxin the implications for sampling and the detection.	JRC and FR input on cyanobacteria given at Nov Stakeholders meeting
• JRC sought clarification that very few epidemiological data for cyanobacteria were available from Europe while we could find many from USA	Noted and clarified with JRC
Epidemiology	
• JRC requested greater clarity in presentation of studies from marine site	Noted
Approved Methods	
• EMEG noted that the example of the Catalan area is not the only area in Europe where this modified method are used and hence should be uses as an example only.	Example deleted in updated background document
• EMEG reviewer noted Colilert® method is standardized as ISO 9308-2 for specific submissions.	Background document amended
New Methods	
• FR noted PCR is a very interesting and innovative tool for the investigation of outbreak but not ready for use in routine monitoring due to use with cost and accreditation.	Key aspect for consideration at WHO WQTAG
Sanitary surveys and bathing water profiling	
• EMEC agree that for the development of sanitary profiles QMRA and MST are valuable tools and should be considered in the future directive.	Noted
Other parameters and Emerging issues	
• EMEG noted that Fujioka et al suggests other parameters that should be reflected here. Fujioka point to: ○ *perfringens, coliphages, and Bacteroides but notes reliability of monitoring for alternative sewage markers was not determined* ○ *and notes beach sand is an unregulated source of FIB and pathogens*	Background document amended and consider covering in emerging and wider issues

• EMEG noted that in times of antimicrobial resistance and wide-spreading of endemic microbe we should be considering the health protection of recreational water users as a whole and go beyond GI illness	Aspect for consideration at WHO WQTAG
FR noted others pathogens such as parasites (crypto or giardia) or on amoebae are not discussed.	FIO, viruses and HAB selected in screening phase (A) for in depth review.
Classification system	
• EMEG noted great discussion is needed on sample numbers and time and the effect of clarification.	Key aspect for consideration at WHO WQTAG

Abbreviations

AdV	adenovirus
E. coli	*Escherichia coli*
EMEG	European Microbiology Expert Group
ENT	enterococci
FIO	faecal indicator organism
FR	France
GL	Guidelines
HAB	harmful algal bloom
JRC	Joint Research Centre
MST	microbial source tracking
PCR	polymerase chain reaction
QMRA	quantitative microbial risk assessment
WHO	World Health Organization
WQTAG	water quality technical advisory group