Draft for consultation

The views expressed in the document do not necessarily represent the views of the European Commission.

Bathing Water Directive ***draft*** parameter fact sheets

The European Commission (EC) is required to review the current Bathing Water Directive (BWD) – Directive 2006/7/EC, 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 revised Directive. The first three parameters aim to provide an indication of faecal contamination, while the organisms that cause HABs are indigenous to the water and present a more natural hazard.

As noted by the World Health Organization[1] (WHO), there are a number of ideal characteristics for a 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.*"

These factsheets are based on a rapid review of the scientific literature conducted during 2017 to update the relevant information in the WHO 2003 Guidelines for Safe Recreational Water Environments[1] (chapters 7 and 8) and the 2009 Addendum to the Guidelines[2] (chapter 4). The final version will represent the advice of the WHO and will incorporate aspects for consideration in an

[1] WHO (2003) Guidelines for safe recreational water environments. Volume 1: Coastal and fresh waters. World Health Organization, Geneva, Switzerland.
[2] 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.

update to the BWD, based on the discussions of the WHO Water Quality and Health Technical Advisory Group (scheduled for January 2018).

A. Current parameter – intestinal enterococci

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.

Their use, however, is not free from confounders and it is known that a number of environmental habitats can serve as both sources and sinks of ENT and studies have shown that populations of ENT may be endogenous in sediments and soils and not exclusively of faecal origin [(1)].

A1. 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 Bathing Water Directive (EU BWD) and a number of other recreational water regulations throughout the world (although many of these regulations are currently under review), outlined in Table A1. The EU BWD is the only set of major regulations that requires the measurement of both *Escherichia coli* (*E. coli*) and ENT at monitored sites.

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 1280 CCE (single value)	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)	Based on 'sufficient' classification Based on 'good' classification Measurements for *E. coli* also required	R	EU [(5)]
Marine	≤185 cfu (90-percentile) ≤200 cfu (95-percentile)	Based on 'sufficient' classification Based on 'good' 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 CCE: calibrator cell equivalents GI: gastrointestinal
USEPA: United States Environmental Protection Agency

A2. 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 review focuses on that which was used to inform the WHO Guidelines [3,7], large European studies and work published since 2009.

The microbial water quality figures 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 water quality ascribed to individual bathers, thus giving an accurate assessment of exposure. Only ENT (measured as faecal streptococci) measured at chest depth showed a 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 at 60/100ml or above). 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 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 incremental elevation in illness as the exposure increased.

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 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].

In addition to the studies outlined in the Table, a number of combined analyses have also been performed. Skin symptoms in swimmers versus non-swimmers at FIO level 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.

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

A3. 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 is a single recreational water regulatory approved qPCR method (ENT: Method 1611 [27]) and the use of this method was supported by the results of epidemiological studies conducted in the USA (at sewage-impacted beaches) which showed a relationship between bather health and ENT measured using qPCR [12, 28].

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].

The EU BWD stipulated a choice of two International Organization for Standardization (ISO) methods, based on culture techniques, for ENT (ISO 7899-1 [30] and 7899-2 [31]). Member States can, however, use alternative methods providing that the alternative method's equivalence to the reference method is demonstrated.

The methods [30, 31] 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 [31] 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 two methods [27, 32] for the enumeration of ENT in recreational waters using qPCR (Method 1611 and Method 1609). In Method 1611 (the method stipulated in the 2012 USEPA regulations [4]), ENT target-DNA sequences present in the sample (based on a specific region of the large subunit ribosomal RNA) are detected by qPCR using TaqMan® '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 100ml. 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 programmes [4].

Method 1609 uses an 'environmental master mix' and also includes an internal amplification control but is, otherwise, similar to Method 1611. 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.

A4. Bathing water profile

Bathing waters designated under the EU BWD require a bathing water profile (Annex III). This includes identification and assessment of pollution (and its causes) that could impact on the 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 can also be used 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).

A4.1 MST

The idea behind MST is that certain faecal microbes are strongly associated with specific hosts (e.g. humans) and that certain identified attributes of those microbes can be used as markers for faecal contamination from that host [34]. Table A3 shows some of the MST targets and 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		
Human polyomavirus - HPyV	Bovine AdV - BAdV		
HF183	Bovine PyV - BPyV		
BacHum	Pig2Bac		
HumM2	Porcine AdV - PAdV		
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].

While the concept behind source tracking is conceptually clear, the application of techniques and interpretation of results is work in progress [38]. Ideally, source apportionment would allow just that, the knowledge that (say) 75% of FIO are derived from human sources, 15% from gulls and up to 10% from dogs and other unspecified sources. Unfortunately, such quantification currently relies on a number of assumptions [38-40], which are either untrue and/or 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 proportion is similar between species and markers;
- each host-specific marker has a similar prevalence and proportional distribution among individuals within the species;
- the host-specific markers are correlated with conventional FIO.

A4.2 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 [41] and to improve routine bathing water monitoring and management [42]. Some of the questions posed by recent recreational water QMRAs include [41, 43-51]:

- 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 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 (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?

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 trailed in Canada where, in the absence of human or bovine MST markers, QMRA may be used to develop site-specific faecal indicator levels.

A5. Prediction 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 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. 52-59] including Multiple Linear Regression (MLR), Artificial Neural Networks (ANN), decision tree and hydrodynamic modelling, with MLR being the most commonly applied. To be useful management tools predictive models should achieve an explained variance (R^2 value) of >60% with well

documented control of multicollinearity [60]. 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 [61], was recommended [60]; 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 [62, 63].

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 [64, 65]. Simple rainfall thresholds were investigated in the development of early UK prediction of bathing water quality [66]. However, it is generally true that the drivers of FIO concentration in recreational waters are more complex than can be characterised by a single driver. 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, England, Wales and Portugal [66, 67]) 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 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 [68, 69]. 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.

Although modelling costs (especially where data acquisition 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.

A6. Classification

The current 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 if agreed) and should consist of at least 16 samples. Samples are taken, immediately before and then, at least monthly, throughout the bathing season.

Table A4: Bathing Water Directive standards for recreational water and classification results for 2015 & 2016 [5, 70, 71]

Parameter	Excellent quality	Good quality	Sufficient
Inland waters			
ENT (cfu/100ml)	200 (*)	400 (*)	330 (**)
E. coli (cfu/100ml)	500 (*)	1000 (*)	900 (**)
Coastal and 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
2015 classification (%)	84.4	9.1	2.6

(*) based upon a 95-percentile evaluation (**) based upon a 90-percentile evaluation

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, taking a closure sample (not to be used for classification), the resampling (if required) of the affected bathing water and a stipulation that no more than one sample per year or no more than 15% of samples from the four-year water quality classification record (whichever is greater) fall into this category.

It can be seen from Table A4 that the majority of 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. Countries reported an almost equal split between ENT and *E. coli* levels for fresh water classification.

Results from the 2016 bathing season monitor 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 [72].

A7. Aspects for consideration for BWD revision

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 factsheet, however, is solely on health protection.

ENT meets many of the requirements for a regulatory parameter of public health significance [3] and it is felt that it should be retained within the BWD, within the current classification system (i.e. the 'sufficient' category should be retained). It is suggested, however, that there are a number of aspects that require consideration:

- Higher ENT concentration allowable at inland sites (i.e. is it appropriate?)

- Sample numbers (a minimum of only 16 collected over a 4 year period – is that acceptable?)
- Guidance on unacceptably high concentrations (e.g. similar to USEPA beach action values) – maybe not to define a legal classification point of view but to inform management decisions.
- What is the best way to protect public health – a long term classification or appropriate information upon which to base day to day decisions (or is a combination of both required)?

A8. References

1. Byappanahalli *et al*. (2012) Enterococci in the environment. *Microbiology and Molecular Biology Reviews* 76(4): 685–706.
2. Wright *et al*. (2009) Microbial load from animal feces at a recreational beach. *Marine Pollution Bulletin* 58(11): 1649–1656.
3. WHO (2003) Guidelines for Safe Recreational Water Environments. Volume 1: Coastal and Fresh Waters. World Health Organization, Geneva, Switzerland.
4. USEPA (2012a) Recreational water quality criteria. Office of Water 820-F-12-058. United States Environmental Protection Agency: Washington, D.C., USA.

5 EU (2006) Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. Official Journal of the European Union L 64.

6. Health Canada (2012) Guidelines for Canadian recreational water quality. Third Edition. Health Canada, Ottawa, Canada.
7. 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.
8. Kay *et al*. (1994) Predicting likelihood of gastroenteritis from sea bathing: results from randomised exposure. *The Lancet* 344: 905–909.
9. Fleisher *et al.* (1996) Marine waters contaminated with domestic sewage: nonenteric illnesses associated with bather exposure in the United Kingdom. *American Journal of Public Health* 86(9): 1228–1234.
10. Wiedenmann *et al*. (2006) A randomized controlled trial assessing infectious disease risks from bathing in fresh recreational waters in relation to the concentration of *Escherichia coli*, intestinal enterococci, *Clostridium perfringens*, and somatic coliphages. *Environmental Health Perspectives* 114: 228-236.
11. Epibathe (2009) Accessible report to Commission staff responsible for reporting to the Parliament and to the Council under Article 14 of the Bathing Water Directive of 15th February 2006. Project reference 022618.
12. Wade *et al*. (2010) Rapidly measured indicators of recreational water quality and swimming-associated illness at marine beaches: a prospective cohort study. *Environmental Health* 9: 66.
13. Fleisher *et al*. (2010) The BEACHES Study: health effects and exposures from non-point source microbial contaminants in subtropical recreational marine waters. *International Journal of Epidemiology* 39: 1291–1298.
14. Colford *et al*. (2012) Using rapid indicators for enterococcus to assess the risk of illness after exposure to urban runoff contaminated marine water. *Water Research* 46(7): 2176–2186.
15. Arnold *et al*. (2013) Swimmer illness associated with marine water exposure and water quality indicators: impact of widely used assumptions. *Epidemiology* 24: 845–853.
16. Yau *et al*. (2014) Effect of submarine groundwater discharge on bacterial indicators and swimmer health at Avalon Beach, CA, USA. *Water Research* 59: 23–36.
17. Arnold *et al*. (2017) Acute illness among surfers after exposure to seawater in dry- and wet-weather conditions. *American Journal of Epidemiology* 186: 866-975
18. Papastergiou *et al*. (2011) Bather density as a predominant factor for health effects related to recreational bathing: Results from the Greek bathers cohort study. *Marine Pollution Bulletin* 62(3): 590–595.
19. Papastergiou *et al*. (2012) Elevated bathing-associated disease risks despite certified water quality: a cohort study. *International Journal of Environmental Research and Public Health* 9: 1548–1565.
20. Harder-Lauridsen *et al*. (2013) Gastrointestinal illness among triathletes swimming in non-polluted versus polluted seawater affected by heavy rainfall, Denmark, 2010-2011. *PLoS ONE* 8(11).
21. Dorevitch *et al*. (2012) Health risks of limited-contact water recreation. *Environmental Health Perspectives* 120: 192–197.
22. Dorevitch *et al*. (2015) Water quality as a predictor of gastrointestinal illness following incidental contact water recreation. *Water Research* 83: 94–103.
23. Joosten *et al*. (2017) Risk factors for gastroenteritis associated with canal swimming in two cities in the Netherlands during the summer of 2015: A prospective study. *PLoS ONE* 12(4).
24. Yau *et al*. (2009) Skin-related symptoms following exposure to recreational water: a systematic review and meta-analysis. *Water Quality, Exposure and Health* 1(2): 79–103.
25. Arnold *et al*. (2016) Acute gastroenteritis and recreational water: highest burden among young US children. *American Journal of Public Health* 106(9): 1690–1697.
26. Benjamin-Chung *et al*. (2017) Coliphages and gastrointestinal illness in recreational waters: pooled analysis of six coastal beach cohorts. *Epidemiology* 28: 644-652.
27. USEPA (2012) Method 1611: Enterococci in water by TaqMan® quantitative polymerase chain reaction (qPCR) assay. Office of Water, EPA-821-R-12-008. United States Protection Agency: Washington, D.C., USA.

28. Wade *et al*. (2008) High sensitivity of children to swimming-associated gastrointestinal illness. Results using a rapid assay of recreational water quality. *Epidemiology* 19: 375-383.
29. Tallon *et al.* (2005) Microbial indicators of faecal contamination in water: a current perspective. *Water, Air, and Soil Pollution* 166(1–4): 139–166.
30. ISO (1998) Water quality – Detection and enumeration of intestinal enterococci in surface and waste water; Part 1: Miniaturized method (Most Probable Number) for surface and waste water. ISO 7899-1:1998. International Organization for Standardization, Switzerland.
31. ISO (2000) Water quality – Detection and enumeration of intestinal enterococci in surface and waste water; Part 2: Method by membrane filtration. ISO 7899-2:2000. International Organization for Standardization, Switzerland.
32. USEPA (2013) Method 1609: Enterococci in water by TaqMan® quantitative polymerase chain reaction (qPCR) with internal amplification control (IAC) assay. Office of Water, EPA-820-R-13-005. United States Protection Agency: Washington, D.C., USA.
33. Sivaganensan *et al*. (2014) Comparison of Enterococcus quantitative polymerase chain reaction analysis results from Midwest U.S. river samples using EPA Method 1611 and Method 1609 PCR reagents. *Journal of Microbiological Methods* 101: 9–17.
34. Harwood *et al*. (2014) Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. *FEMS Microbiology Reviews* 38(1): 1–40.
35. Kinzelman & McLellan (2009) Success of science-based best management practices in reducing swimming bans—a case study from Racine, Wisconsin, USA. *Aquatic Ecosystem Health & Management* 12(2): 187–196.
36. Walker *et al*. (2015) A tiered approach for the identification of faecal pollution sources on an Auckland urban beach. *New Zealand Journal of Marine and Freshwater Research* 49(3): 333–345.
37. Goodwin *et al*. (2016) Watershed assessment with beach microbial source tracking and outcomes of resulting gull management. *Environmental Science & Technology* 50(18): 9900–9906.
38. Kinzelman *et al*. (2012) Indicators, sanitary surveys and source attribution techniques. In: Animal Waste, Water Quality and Human Health, Eds: Dufour A, Bartram J, Bos R, Gannon V. IWA Publishing, London.
39. Field & Samadpour (2007) Fecal source tracking, the indicator paradigm, and managing water quality. *Water Research* 41(16): 3517–3538.
40. Roslev & Bukh (2011) State of the art molecular markers for fecal pollution source tracking in water. *Applied Microbiology and Biotechnology* 89(5): 1341–1355.
41. Fewtrell & Kay (2015) Recreational water and infection: A review of recent findings. *Current Environmental Health Reports* 2(1): 85–94.
42. Ashbolt *et al*. (2010) Predicting pathogen risks to aid beach management: The real value of quantitative microbial risk assessment (QMRA). *Water Research* 44(16): 4692–4703.
43. Schoen *et al*. (2011) Evaluating the importance of faecal sources in human-impacted waters. *Water Research* 45: 2670-2680.
44. Soller *et al*. (2014) Human health risk implications of multiple sources of faecal indicator bacteria in a recreational waterbody. *Water Research* 66: 254–264.
45. Rijal *et al.* (2011) Microbial risk assessment for recreational use of the Chicago Area Waterway System. *Journal of Water and Health* 9(1): 169–186.
46. Júlio *et al*. (2012) Waterborne transmission of *Giardia* and *Cryptosporidium* at river beaches in Southern Europe (Portugal). *Journal of Water and Health* 10(3): 484–496.
47. Soller *et al*. (2015) Estimated human health risks from recreational exposure to stormwater runoff containing animal fecal material. *Environmental Modelling & Software* 72: 21-32.
48. Soller *et al*. (2017) Incidence of gastrointestinal illness following wet weather recreational exposures: Harmonization of quantitative microbial risk assessment with an epidemiologic investigation of surfers. *Water Research* 121: 280-289.
49. Lim *et al*. (2017) Evaluation of the dry and wet weather recreational health risks in a semi-enclosed marine embayment in Southern California. *Water Research* 111: 318–329.
50. Eregno *et al*. (2016) Quantitative microbial risk assessment combined with hydrodynamic modelling to estimate the public health risk associated with bathing after rainfall events. *Science of the Total Environment* 548–549: 270–279.
51. Boehm *et al*. (2015) Human-associated and fecal quantitative polymerase chain reaction measurements and simulated risk of gastrointestinal illness in recreational waters contaminated with raw sewage. *Environmental Science & Technology Letters* 2: 270-275.
52. Dada & Hamilton (2016) Predictive models for determination of *E. coli* concentrations at inland recreational beaches. *Water Air and Soil Pollution* 227: 347.
53. Francy *et al*. (2013) Predictive models for *Escherichia coli* concentrations at inland lake beaches and relationship of model variables to pathogen detection. *Applied and Environmental Microbiology* 79(5): 1676-1688.
54. Ge & Frick (2009) Time-frequency analysis of beach bacteria variations and its implication for recreational water quality modelling. *Environmental Science and Technology* 43: 1128–1133.
55. McPhail & Stidson (2009) Bathing water signage and predictive water quality models in Scotland. *Aquatic Ecosystem Health and Management* 12(2): 183–186.
56. Nevers *et al*. (2014) Beach science in the Great Lakes. *Journal of Great Lakes Research* 40(1): 1–14.
57. Nevers & Whitman (2011) Efficacy of monitoring and empirical predictive modeling at improving public health protection at Chicago beaches. *Water Research* 45(4): 1659–1668.

58. Nevers & Whitman (2010) Policies and practices of beach monitoring in the Great Lakes, USA: a critical review. *Journal of Environmental Monitoring: JEM* 12(3): 581–590.
59. Stidson *et al*. (2012) Development and use of modelling techniques for real-time bathing water quality predictions. *Water Environment Journal* 26(1): 7–18.
60. WHO (1999) Health based monitoring of recreational waters: the feasibility of a new approach; the 'Annapolis Protocol'. World Health Organization, Geneva, Switzerland.
61. Stapleton *et al*. (2009) Evaluating the operational utility of a *Bacteroidales* quantitative PCR-based MST approach in determining the source of faecal indicator organisms at a UK bathing water. *Water Research* 43(19): 4888–4899.
62. Bedri *et al*. (2016) Evaluating a microbial water quality prediction model for beach management under the revised EU Bathing Water Directive. *Journal of Environmental Management* 167: 49–58.
63. Karlsson & Liungman (2010) Bathing water forecasts for the general public—coupling urban, riverine and coastal operational modelling of bacteria. International MIKE by DHI Conference, DHI, Denmark.
64. Paule *et al*. (2015) Faecal contamination in Yongin watershed: association to land use and land cover and stormwater quality. *Desalination and Water Treatment* 53(11): 3026–3038.
65. Wyer *et al*. (2010) Evaluating short-term changes in recreational water quality during a hydrograph event using a combination of microbial tracers, environmental microbiology, microbial source tracking and hydrological techniques: A case study in Southwest Wales, UK. *Water Research* 44(16): 4783–4795.
66. Dhondia *et al*. (2014) Prediction and dissemination of bathing water quality In England and Wales – A pilot study (2014). *CUNY Academic Works.*
67. Viegas *et al*. (2009) Streams contribution on bathing water quality rainfall events in Costa do Estoril- a tool to implement an alert system for the bathing water quality. *Journal of Coastal Research* SI56: 1691 - 1695.
68. Boehm *et al*. (2002) Decadal and shorter period variability of surf zone water quality at Huntington Beach, California. *Environmental Science & Technology* 36(18): 3885-3892.
69. Boehm & Weisberg (2005) Tidal forcing of enterococci at marine recreational beaches at fortnightly and semidiurnal frequencies. *Environmental Science & Technology* 39: 5575-5583.
70. EEA (2016) EEA (2016) European bathing water quality in 2015. EEA Report 9/2016. European Environment Agency, Denmark.
71. EEA (2017) EEA (2017) European bathing water quality in 2016. EEA Report 5/2017. European Environment Agency, Denmark.
72. Quilliam *et al*. (2016) Resolving conflicts in public health protection and ecosystem service provision at designated bathing waters. *Journal of Environmental Management* 161: 237–242.

B. Current parameter - *Escherichia coli*

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 present in greater numbers than enterococci (ENT).

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)].

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.

B1. Current situation

E. coli is currently used as a regulatory parameter in both the European Union Bathing Water Directive (EU BWD) and a number of other recreational water regulations throughout the world (although many of these regulations are currently under review), as summarised in Table B1. The EU BWD is the only set of major regulations that requires the measurement of both *E. coli* and ENT at all monitored sites.

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)	Based on 'sufficient' classification Based on 'good' classification Measurements for ENT also required	R	EU [(6)]
Marine	≤500 cfu (90-percentile) ≤500 cfu (95-percentile)	Based on 'sufficient' classification Based on 'good' 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

B2. 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 water quality ascribed to individual bathers, thus giving an accurate assessment of exposure. Only ENT (measured as faecal streptococci) measured at chest depth showed a 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 at 60/100ml or above). 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 incremental elevation in illness as the exposure increased.

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 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 levels of *E. coli*. Where a relationship has been suggested [19, 20], there is inadequate information to derive a dose-response.

In addition to the studies outlined in the Table, a combined analysis of a number of studies has been performed. Skin symptoms in swimmers versus non-swimmers at FIO level 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

B3. 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 is a single recreational water regulatory approved qPCR method (ENT: Method 1611 [25]).

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 EU 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 EU BWD for *E. coli* are ISO 9308-1 [26] and 9308-3 [27]; 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 [28]. ISO 9308-2: 2012 [29] was not available when the BWD was published. 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 [28].

B4. Bathing water profile

Bathing waters designated under the EU BWD require a bathing water profile (Annex III). This includes identification and assessment of pollution (and its causes) that could impact on the 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 can also be used 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).

B4.1 MST

The idea behind MST is that certain faecal microbes are strongly associated with specific hosts (e.g. humans) and that certain identified attributes of those microbes can be used as markers for faecal

contamination from that host [30]. Table B3 shows some of the MST targets and 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		
Human polyomavirus - HPyV	Bovine AdV - BAdV		
HF183	Bovine PyV - BPyV		
BacHum	Pig2Bac		
HumM2	Porcine AdV - PAdV		
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 [31-33].

While the concept behind source tracking is conceptually clear, the application of techniques and interpretation of results is work in progress [34]. Ideally, source apportionment would allow just that, the knowledge that (say) 75% of FIO are derived from human sources, 15% from gulls and up to 10% from dogs and other unspecified sources. Unfortunately, such quantification currently relies on a number of assumptions [34-36], which are either untrue and/or 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 proportion is similar between species and markers;
- each host-specific marker has a similar prevalence and proportional distribution among individuals within the species;
- the host-specific markers are correlated with conventional FIO.

B4.2 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 [37] and to improve routine bathing water monitoring and management [38]. Some of the questions posed by recent recreational water QMRAs include [37. 39-47]:

- 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 (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?

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 trailed in Canada where, in the absence of human or bovine MST markers, QMRA can be used to develop site-specific faecal indicator levels.

B5. Prediction 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 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. 48-55] including Multiple Linear Regression (MLR), Artificial Neural Networks (ANN), decision tree and hydrodynamic modelling, with MLR being the most commonly applied. To be useful management tools, predictive models should achieve a high explained variance (R^2 value) possibly >60% with well documented control of multicollinearity [56]. 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 [57], was recommended [56]; 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 [58, 59].

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 [60, 61]. Simple rainfall thresholds were investigated in the development of early UK prediction of bathing water quality [62]. However, it is generally true that the drivers of FIO concentration in recreational waters are more complex than can be characterised by a single driver. 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, England, Wales and Portugal [62, 63]) 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 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 [64, 65]. 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.

Although modelling costs (especially where data acquisition 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.

B6. 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. Samples are taken, immediately before and then, at least monthly, throughout the bathing season.

Table B4: Bathing Water Directive standards for recreational water and classification results for 2015 & 2016 [6, 66, 67]

Parameter	Excellent quality	Good quality	Sufficient
Inland waters			
ENT (cfu/100ml)	200 (*)	400 (*)	330 (**)
E. coli (cfu/100ml)	500 (*)	1000 (*)	900 (**)
Coastal and 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
2015 classification (%)	84.4	9.1	2.6

(*) based upon a 95-percentile evaluation (**) based upon a 90-percentile evaluation

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, taking a closure sample (not to be used for classification), the resampling (if required) of the affected bathing water and a stipulation that no more than one sample per year or no more than 15% of samples from the four-year water quality classification record (whichever is greater) fall into this category.

It can be seen from Table B4 that the majority of 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. Countries reported an almost equal split between ENT and *E. coli* levels for fresh water classification.

Results from the 2016 bathing season monitor 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 [(68)].

B7. Aspects for consideration for BWD revision

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 factsheet, however, is solely on health protection.

E. coli meets a number of the requirements for a regulatory parameter of public health significance [(7)], although there is a lack of convincing epidemiological data to drive guideline or regulatory values especially in marine waters. This, evidence deficit is the key area which requires consideration.

B8. References

1. Tallon *et al.* (2005) Microbial indicators of faecal contamination in water: a current perspective. *Water, Air, and Soil Pollution* 166(1–4): 139–166.
2. Croxen *et al*. (2013) Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clinical Microbiology Reviews* 26(4): 822–880.
3. Luo *et al*. (2011) Genome sequencing of environmental *Escherichia coli* expands uunderstanding of the ecology and speciation of the model bacterial species. *Proceedings of the National Academy of Science USA* 108: 7200-7205.
4. USEPA (2012) Recreational water quality criteria. Office of Water 820-F-12-058. United States Environmental Protection Agency: Washington, D.C., USA.
5. Health Canada Guidelines for Canadian recreational water quality. Third Edition. Health Canada, Ottawa, Canada.
6. EU (2006) Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. Official Journal of the European Union L 64.
7. WHO (2003) Guidelines for Safe Recreational Water Environments. Volume 1: Coastal and Fresh Waters. World Health Organization, Geneva, Switzerland.
8. 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.
9. Kay *et al*. (1994) Predicting likelihood of gastroenteritis from sea bathing: results from randomised exposure. *The Lancet* 344: 905–909.
10. Fleisher *et al.* (1996) Marine waters contaminated with domestic sewage: nonenteric illnesses associated with bather exposure in the United Kingdom. *American Journal of Public Health* 86(9): 1228–1234.
11. Wiedenmann *et al*. (2006) A randomized controlled trial assessing infectious disease risks from bathing in fresh recreational waters in relation to the concentration of *Escherichia coli*, intestinal enterococci, *Clostridium perfringens*, and somatic coliphages. *Environmental Health Perspectives* 114: 228-236.
12. Epibathe (2009) Accessible report to Commission staff responsible for reporting to the Parliament and to the Council under Article 14 of the Bathing Water Directive of 15th February 2006. Project reference 022618.
13. Colford *et al*. (2012) Using rapid indicators for enterococcus to assess the risk of illness after exposure to urban runoff contaminated marine water. *Water Research* 46(7): 2176–2186.

14. Arnold *et al*. (2013) Swimmer illness associated with marine water exposure and water quality indicators: impact of widely used assumptions. *Epidemiology* 24: 845–853.
15. Yau *et al*. (2014) Effect of submarine groundwater discharge on bacterial indicators and swimmer health at Avalon Beach, CA, USA. *Water Research* 59: 23–36.
16. Arnold *et al*. (2017) Acute illness among surfers after exposure to seawater in dry- and wet-weather conditions. *American Journal of Epidemiology* 186: 866-975
17. Papastergiou *et al*. (2011) Bather density as a predominant factor for health effects related to recreational bathing: Results from the Greek bathers cohort study. *Marine Pollution Bulletin* 62(3): 590–595.
18. Papastergiou *et al*. (2012) Elevated bathing-associated disease risks despite certified water quality: a cohort study. *International Journal of Environmental Research and Public Health* 9: 1548–1565.
19. Harder-Lauridsen *et al*. (2013) Gastrointestinal illness among triathletes swimming in non-polluted versus polluted seawater affected by heavy rainfall, Denmark, 2010-2011. *PLoS ONE* 8(11).
20. Marion *et al*. (2010) Association of gastrointestinal illness and recreational water exposure at an inland U.S. beach. *Water Research* 44: 4796–4804.
21. Dorevitch *et al*. (2012) Health risks of limited-contact water recreation. *Environmental Health Perspectives* 120: 192–197.
22. Dorevitch *et al*. (2015) Water quality as a predictor of gastrointestinal illness following incidental contact water recreation. *Water Research* 83: 94–103.
23. Joosten *et al*. (2017) Risk factors for gastroenteritis associated with canal swimming in two cities in the Netherlands during the summer of 2015: A prospective study. *PLoS ONE* 12(4).
24. Yau *et al*. (2009) Skin-related symptoms following exposure to recreational water: a systematic review and meta-analysis. *Water Quality, Exposure and Health* 1(2): 79–103.
25. USEPA (2012) Method 1611: Enterococci in water by TaqMan® quantitative polymerase chain reaction (qPCR) assay. Office of Water, EPA-821-R-12-008. United States Protection Agency: Washington, D.C., USA.
26. ISO (2014) Water quality – Enumeration of Escherichia coli and coliform bacteria; Part 1: Membrane filtration method for waters with low bacterial background flora. ISO 9308-1:2014. International Organization for Standardization, Switzerland.
27. ISO (1998) Water quality – Enumeration of Escherichia coli and coliform bacteria; Part 3: Miniaturized method (Most Probable Number) for the detection and enumeration of E.coli in surface and waste water. ISO 9308-3:1998. International Organization for Standardization, Switzerland.
28. EMEG (2016) Current state of enumeration methods for *Escherichia coli* in regulatory bathing water quality monitoring in Europe. EMEG Outline paper; European Microbiology Bathing Water Sub-Expert Group. September 2016.
29. ISO (2012) Water quality – Enumeration of Escherichia coli and coliform bacteria; Part 2: Most probable number method. ISO 9308-2:2012. International Organization for Standardization, Switzerland.
30. Harwood *et al*. (2014) Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. *FEMS Microbiology Reviews* 38(1): 1–40.
31. Kinzelman & McLellan (2009) Success of science-based best management practices in reducing swimming bans—a case study from Racine, Wisconsin, USA. *Aquatic Ecosystem Health & Management* 12(2): 187–196.
32. Walker *et al*. (2015) A tiered approach for the identification of faecal pollution sources on an Auckland urban beach. *New Zealand Journal of Marine and Freshwater Research* 49(3): 333–345.
33. Goodwin *et al*. (2016) Watershed assessment with beach microbial source tracking and outcomes of resulting gull management. *Environmental Science & Technology* 50(18): 9900–9906.
34. Kinzelman *et al*. (2012) Indicators, sanitary surveys and source attribution techniques. In: Animal Waste, Water Quality and Human Health, Eds: Dufour A, Bartram J, Bos R, Gannon V. IWA Publishing, London.
35. Field & Samadpour (2007) Fecal source tracking, the indicator paradigm, and managing water quality. *Water Research* 41(16): 3517–3538.
36. Roslev & Bukh (2011) State of the art molecular markers for fecal pollution source tracking in water. *Applied Microbiology and Biotechnology* 89(5): 1341–1355.
37. Fewtrell & Kay (2015) Recreational water and infection: A review of recent findings. *Current Environmental Health Reports* 2(1): 85–94.
38. Ashbolt *et al*. (2010) Predicting pathogen risks to aid beach management: The real value of quantitative microbial risk assessment (QMRA). *Water Research* 44(16): 4692–4703.
39. Schoen *et al*. (2011) Evaluating the importance of faecal sources in human-impacted waters. *Water Research* 45: 2670-2680.
40. Soller *et al*. (2014) Human health risk implications of multiple sources of faecal indicator bacteria in a recreational waterbody. *Water Research* 66: 254–264.
41. Rijal *et al.* (2011) Microbial risk assessment for recreational use of the Chicago Area Waterway System. *Journal of Water and Health* 9(1): 169–186.
42. Júlio *et al*. (2012) Waterborne transmission of *Giardia* and *Cryptosporidium* at river beaches in Southern Europe (Portugal). *Journal of Water and Health* 10(3): 484–496.
43. Soller *et al*. (2015) Estimated human health risks from recreational exposure to stormwater runoff containing animal fecal material. *Environmental Modelling & Software* 72: 21-32.

44. Soller *et al*. (2017) Incidence of gastrointestinal illness following wet weather recreational exposures: Harmonization of quantitative microbial risk assessment with an epidemiologic investigation of surfers. *Water Research* 121: 280-289.
45. Lim *et al*. (2017) Evaluation of the dry and wet weather recreational health risks in a semi-enclosed marine embayment in Southern California. *Water Research* 111: 318–329.
46. Eregno *et al*. (2016) Quantitative microbial risk assessment combined with hydrodynamic modelling to estimate the public health risk associated with bathing after rainfall events. *Science of the Total Environment* 548–549: 270–279.
47. Boehm *et al*. (2015) Human-associated and fecal quantitative polymerase chain reaction measurements and simulated risk of gastrointestinal illness in recreational waters contaminated with raw sewage. *Environmental Science & Technology Letters* 2: 270-275.
48. Dada & Hamilton (2016) Predictive models for determination of *E. coli* concentrations at inland recreational beaches. *Water Air and Soil Pollution* 227: 347.
49. Francy *et al*. (2013) Predictive models for *Escherichia coli* concentrations at inland lake beaches and relationship of model variables to pathogen detection. *Applied and Environmental Microbiology* 79(5): 1676-1688.
50. Ge & Frick (2009) Time-frequency analysis of beach bacteria variations and its implication for recreational water quality modelling. *Environmental Science and Technology* 43: 1128–1133.
51. McPhail & Stidson (2009) Bathing water signage and predictive water quality models in Scotland. *Aquatic Ecosystem Health and Management* 12(2): 183–186.
52. Nevers *et al*. (2014) Beach science in the Great Lakes. *Journal of Great Lakes Research* 40(1): 1–14.
53. Nevers & Whitman (2011) Efficacy of monitoring and empirical predictive modeling at improving public health protection at Chicago beaches. *Water Research* 45(4): 1659–1668.
54. Nevers & Whitman (2010) Policies and practices of beach monitoring in the Great Lakes, USA: a critical review. *Journal of Environmental Monitoring: JEM* 12(3): 581–590.
55. Stidson *et al*. (2012) Development and use of modelling techniques for real-time bathing water quality predictions. *Water Environment Journal* 26(1): 7–18.
56. WHO (1999) Health based monitoring of recreational waters: the feasibility of a new approach; the 'Annapolis Protocol'. World Health Organization, Geneva, Switzerland.
57. Stapleton *et al*. (2009) Evaluating the operational utility of a *Bacteroidales* quantitative PCR-based MST approach in determining the source of faecal indicator organisms at a UK bathing water. *Water Research* 43(19): 4888–4899.
58. Bedri *et al*. (2016) Evaluating a microbial water quality prediction model for beach management under the revised EU Bathing Water Directive. *Journal of Environmental Management* 167: 49–58.
59. Karlsson & Liungman (2010) Bathing water forecasts for the general public—coupling urban, riverine and coastal operational modelling of bacteria. International MIKE by DHI Conference, DHI, Denmark.
60. Paule *et al*. (2015) Faecal contamination in Yongin watershed: association to land use and land cover and stormwater quality. *Desalination and Water Treatment* 53(11): 3026–3038.
61. Wyer *et al*. (2010) Evaluating short-term changes in recreational water quality during a hydrograph event using a combination of microbial tracers, environmental microbiology, microbial source tracking and hydrological techniques: A case study in Southwest Wales, UK. *Water Research* 44(16): 4783–4795.
62. Dhondia *et al*. (2014) Prediction and dissemination of bathing water quality In England and Wales – A pilot study (2014). *CUNY Academic Works.*
63. Viegas *et al*. (2009) Streams contribution on bathing water quality rainfall events in Costa do Estoril- a tool to implement an alert system for the bathing water quality. *Journal of Coastal Research* SI56: 1691 - 1695.
64. Boehm *et al*. (2002) Decadal and shorter period variability of surf zone water quality at Huntington Beach, California. *Environmental Science & Technology* 36(18): 3885-3892.
65. Boehm & Weisberg (2005) Tidal forcing of enterococci at marine recreational beaches at fortnightly and semidiurnal frequencies. *Environmental Science & Technology* 39: 5575-5583.
66. EEA (2016) EEA (2016) European bathing water quality in 2015. EEA Report 9/2016. European Environment Agency, Denmark.
67. EEA (2017) EEA (2017) European bathing water quality in 2016. EEA Report 5/2017. European Environment Agency, Denmark.
68. Quilliam *et al*. (2016) Resolving conflicts in public health protection and ecosystem service provision at designated bathing waters. *Journal of Environmental Management* 161: 237–242.

C. Possible parameter - viral indicator

Neither *Escherichia coli* (*E. coli*) nor intestinal enterococci (ENT), i.e. the existing parameters specified by the European Union Bathing Water Directive (EU BWD), is considered ideal, especially as the principal cause of bathing-water-acquired illness resulting from faecal contamination is 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.

Adenovirus (AdV): AdV are double-stranded DNA viruses and are members of the Adenoviridae family. They are associated with a range of diseases. In comparison to RNA viruses, they are resistant to environmental inactivation and have been shown to be prevalent worldwide and are shed, asymptomatically, by most 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 (including different types of polioviruses, coxsackieviruses A and B, echoviruses and enteroviruses). 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 genotypes I and II [5] (NoV GI and GII). In a review of viral recreational water outbreaks [4], 25 NoV outbreaks were identified (from 55 outbreaks) of which 16 were associated with lakes (14) or rivers (two), with the other nine outbreaks being attributed to pools (seven), a hot spring and a fountain. 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.

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 similar to AdV [7]. Coliphages are present in sewage and have been isolated from both fresh and marine recreational waters, although sometimes in low numbers.

C1. 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.

C2. 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 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.

The succeeding 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 x 10^6 GC/litre in river water samples and 5 x 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%.

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 x 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 x 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

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

* 95th percentile value of the location with the highest concentration

While the European data for coliphages in recreational water is quite limited, Table C1 suggests that in the surface waters examined, somatic coliphages typically 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.

C3. 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.

C3.1 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 multivariable 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.*"

C3.2 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

C4. 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].

C4.1 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].

C4.2 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. ISO methods) are available for both somatic and F-specific coliphages.

C5. Classification

It is not currently recommended that a viral parameter be included in recreational water guidelines or regulations.

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 a pathogenic virus (e.g. NoV) cannot be taken to infer a lack of 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) prove faecal connectivity to the bathing water.
- Concentrations in recreational waters are often very low, meaning that detection is not straightforward.
- Many of the viral detection methods rely on qPCR methods, which are currently costly and enumerate dead and non-viable material 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.
- 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. References

1. Kauppinen *et al.* (2017) Increase in outbreaks of gastroenteritis linked to bathing water in Finland in summer 2014. *Eurosurveillance* 22(8).
2. NHS (2013) Public Health 2012/2013. The annual report of the Director of Public Health. Lanarkshire NHS Board, Scotland.
3. Zlot *et al*. (2015) Norovirus outbreak associated with a natural lake used for recreation - Oregon, 2014. *American Journal of Transplantation* 15(7): 2001–2005.
4. Sinclair *et al*. (2009) Viruses in recreational water-borne disease outbreaks: A review. *Journal of Applied Microbiology* 107(6): 1769–1780.
5. Patel *et al*. (2009) Noroviruses: A comprehensive review. Journal of Clinical Virology 44(1): 1–8.
6. Ahmed *et al*. (2013) A systematic review and meta-analysis of the global seasonality of norovirus. *PLOS ONE* 8(10): e75922.
7. USEPA (2015) Review of coliphages as possible indicators of fecal contamination for ambient water quality. Office of Water EPA-820-R-15-098. United States Environmental Protection Agency: Washington, D.C., USA.
8. Wyn-Jones *et al*. (2011) Surveillance of adenoviruses and noroviruses in European recreational waters. *Water Research* 45(3): 1025–1038.
9. Kern *et al*. (2013) Detection of enteric viruses in Hungarian surface waters: First steps towards environmental surveillance. *Journal of Water and Health* 11(4): 772–782.
10. Viroclime (2013) Final Report Summary - VIROCLIME (Impact of climate change on the transport, fate and risk management of viral pathogens in water). http://cordis.europa.eu/result/rcn/58568_en.html, accessed 2/8/17
11. Hokajärvi *et al*. (2013) Occurrence of thermotolerant Campylobacter spp. and adenoviruses in Finnish bathing waters and purified sewage effluents. *Journal of Water and Health* 11: 120-134.
12. Silva *et al*. (2010) Viral and bacterial contamination in recreational waters: a case study in the Lisbon bay area. *Journal of Applied Microbiology* 108(3): 1023–1031.
13. Wiedenmann *et al*. (2006) A randomized controlled trial assessing infectious disease risks from bathing in fresh recreational waters in relation to the concentration of *Escherichia coli*, intestinal enterococci, *Clostridium perfringens*, and somatic coliphages. *Environmental Health Perspectives* 114: 228-236.
14. Mocé-Llivina *et al*. (2005) Enteroviruses and bacteriophages in bathing waters. *Applied and Environmental Microbiology* 71(11): 6838–6844.
15. Ibarluzea *et al*. (2007) Somatic coliphages and bacterial indicators of bathing water quality in the beaches of Gipuzkoa, Spain. *Journal of Water and Health* 5(3): 417–426.
16. Bofill-Mas *et al*. (2010) Quantification of human adenoviruses in European recreational waters. *Food and Environmental Virology* 2(2): 101–109.
17. Wyer *et al*. (2012) Relationships between human adenoviruses and faecal indicator organisms in European recreational waters. *Water Research* 46(13): 4130–4141.
18. McMinn *et al*. (2017) Bacteriophages as indicators of faecal pollution and enteric virus removal. *Letters in Applied Microbiology* 65: 11-26.
19. Marion *et al*. (2014) Integrating bacterial and viral water quality assessment to predict swimming-associated illness at a freshwater beach: a cohort study. *PLoS ONE* 9(11).
20. Marion *et al*. (2010) Association of gastrointestinal illness and recreational water exposure at an inland U.S. beach. *Water Research* 44(16): 4796–4804.
21. Von Schirnding *et al*. (1992) Morbidity among bathers exposed to polluted seawater. *South African Medical Journal* 81; 543-546.
22. Colford *et al*. (2007) Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. *Epidemiology* 18: 27-35.
23. Wade *et al*. (2010) Rapidly measured indicators of recreational water quality and swimming-associated illness at marine beaches: a prospective cohort study. *Environmental Health* 9: 66.
24. Abdelzaher *et al*. (2011) Daily measures of microbes and human health at a non-point source marine beach. Journal of *Water and Health* 9(3): 443–457.
25. Griffith *et al*. (2016) Epidemiologic evaluation of multiple alternate microbial water quality monitoring indicators at three California beaches. *Water Research* 94: 371–381.
26. Benjamin-Chung *et al*. (2017) Coliphages and gastrointestinal illness in recreational waters: pooled analysis of six coastal beach cohorts. *Epidemiology* 28: 644-652.
27. Lee *et al*. (1997) Bacteriophages are a better indicator of illness rates than bacteria amongst users of a white water rafting course fed by a lowland river. *Water Science and Technology* 35: 165-170.
28. Van Asperen *et al*. (1998) Risk of gastroenteritis among triathletes in relation to faecal pollution of fresh water. *International Journal of Epidemiology* 27: 309-315.
29. Tallon *et al.* (2005) Microbial indicators of faecal contamination in water: a current perspective. *Water, Air, and Soil Pollution* 166(1–4): 139–166.

30. USEPA (2012) Method 1615: Measurement of enterovirus and norovirus occurrence in water by culture and RT-qPCR. Version 1.1. EPA 600/R-10/181. United States Environmental Protection Agency: Washington, D.C., USA.

D. Exploration of a harmful algal bloom parameter

To follow