European Union Reference Laboratory for monitoring bacteriological and viral contamination of bivalve molluscs

Microbiological Monitoring of Bivalve Mollusc Harvesting Areas

Guide to Good Practice: Technical Application

EU Working Group on the Microbiological Monitoring of Bivalve Mollusc Harvesting Areas

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Revision History

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1	May 2006	
2	September 2006	Table 1.1 amended to remove reference to “Class A areas” with respect to relaying.
3	February 2007	Table 1.1 amended to reflect Regulation (EC) No 1666/2006. Section 3.8 amended to make requirements for the number of samples in remote areas consistent with Section 7.3. Figure 4.1 relabelled Table 4.1. Bibilography section updated with respect to EC Regulations. Changed “Sanitary” to “Shoreline” in title of Annex 1.
4	August 2010	Major revision including: i. Addition of an executive summary ii. Additions to glossary iii. Amendments to some subsections within all sections. iv. Additional Annex
5	June 2014	Executive Summary: sanitary survey review period changed from three to six years to be consistent with main text. Other amendments to reflect changes in the main text. Glossary: definitions of aquaculture, *Escherichia coli*, faecal coliforms, representative sampling point, preliminary (now initial) and initial full (now primary established) classifications, changed to be consistent with the Community Guide; definition of stable area removed to reflect the change to Section 3.11 Section 2.8: reference to the (now repealed) Shellfish Waters Directive changed to the Water Framework Directive. Section 2.14: removal of reference to stability of microbiological monitoring data. Section 2.15: revised to ensure the link

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Guide to Good Practice: Technical Application

Executive Summary

Consumption of raw or insufficiently cooked bivalve molluscs can result in illness due to the presence of microorganisms, many derived from faecal contamination of the bivalves. Within the European Union, food hygiene legislation contains a number of requirements intended to reduce this risk of illness. Those to be undertaken by the Member State competent authorities are given in Regulation (EC) No 854/2004. An evaluation of the sources and potential impact of faecal contamination (both human and animal) in the vicinity of production and relay areas provides the basis for determining the extent of the production area and the sampling plan on which ongoing monitoring is based. Monitoring, using *Escherichia coli* as a faecal indicator organism, then provides an assessment of the risk of contamination with bacterial and viral pathogens. A classification is given to the areas as a result of the assessment and this determines whether the areas can be used for harvesting and what level of post-harvesting treatment is needed to reduce the risk to a level that is regarded as acceptable. Ongoing monitoring determines whether the level of risk has changed and thus whether short-term controls need to be applied or the classification status changed. The application of monitoring programmes has tended to vary significantly between Member States and meetings of the reference laboratory network and the good practice guide was developed in order to provide a common baseline for the protection of public health and promotion of intra-community trade.

The Commission has published a Community Guide which outlines the principles relating to the implementation of Regulation (EC) No 854/2004 with respect to the classification of bivalve mollusc harvesting areas. This Good Practice Guide Technical Application (GPG) document gives recommendations as to how the requirements given in Regulation (EC) No 854/2004, together with the recommendations in the Community Guide, may be achieved in the context of scientific knowledge and experience relating to the conduct of microbiological monitoring programmes. The document covers sanitary surveys, sampling plans, sampling and sample transport, laboratory testing, data handling and storage and interpretation of data.

With respect to sanitary surveys, recommendations are given on the type and detail of information that should be acquired with respect to the potential sources of faecal contamination listed in the legislation. It is also recommended that on-site verification of this information takes place by means of a shoreline survey. Different levels of the determination of the circulation of pollutants are suggested, ranging from simple bathymetry and tidal stream assessment to the use of hydrodynamic and particle

track models. Three different levels of overall assessment are described: qualitative, semi-quantitative and quantitative. These are used to determine the extent to which potential sources of pollution may impact on a harvesting area and thus are used to inform the recommendations from the sanitary survey. It is proposed that, where necessary, some preliminary bacteriological monitoring may also be undertaken. While sanitary surveys are only a legislative requirement for newly classified areas, there is a recommendation that Member States should introduce a programme of work by 1 January 2011 to complete sanitary surveys by 1 January 2015 at the latest. There is also a recommendation that sanitary surveys should be reviewed every six years, with a simple assessment each year as to whether any major changes have occurred in the major contaminating influences.

The outcome of the sanitary survey determines the content of the sampling plan in terms of the number and location of representative sampling points and the frequency and timing of sampling. There are recommendations as to how these should be determined and the information that should be recorded in the sampling plan.

General advice is given on some aspects of sampling and sample transport but recommends that competent authorities should establish protocols for these activities and that sampling officers should be trained and audited. Recommendations are also given on the provision of samples, or results, by the industry.

Microbiological testing is now covered in EU legislation in more detail than before, including the specification of a reference method for *E. coli* in bivalve molluscs (ISO TS 16649-3). The GPG restates some of the requirements that appear in separate pieces of legislation and includes recommendations on dilution ranges to be used for testing. The use of internal quality controls and participation in External Quality Assessment (EQA) schemes and ring trials are also addressed.

Very brief recommendations are given on data handling and storage associated with the microbiological monitoring programme data, largely because this is an area that has not been addressed to date by many Member States and more experience is needed before more detailed recommendations are given, if necessary. It is identified that many elements of information associated with both sanitary surveys and monitoring programme management have geographical components and therefore will benefit from either being stored within a Geographic Information System (GIS) or a GIS-linked database.

The final aspect of a monitoring programme relates to the interpretation of the data. It is recommended that minimum data requirements be applied for the determination and maintenance of classifications in order to ensure that the assessment of risk is based on an adequate data set. In general, for areas with sufficient data, it is recommended that the assessment is based on the last 3 years' data. There are also recommendations for procedures in response to high results and also criteria for deciding whether apparently anomalous results may be disregarded.

The GPG was first published in May 2006. The 2010 update (Issue 4) represented a major revision and incorporates changes in legislation and takes into account

experience gained in application of the Guide. The present revision incorporates some further editorial changes noted during the use of Issue 4, amendments to terminology to be consistent with the Community Guide, and removal of specific criteria for stability that were previously given in Section 3.11, further to a decision taken at the 2012 workshop of National Reference Laboratories. A new Annex (Annex 4) gives guidance relating to additional requirements for production areas from which bivalve molluscs are harvested for export to the USA.

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Glossary

Aquaculture	The rearing or culture of aquatic organisms using techniques designed to increase the production of the organisms in question beyond the natural capacity of the environment, the organisms remaining the property of a natural or legal person throughout the rearing or culture stage, up to and including harvesting (EC 2792/99).[1]
Bacteriological survey	Short-term monitoring undertaken in order to help identify the position(s) for representative sampling point(s) for the classification monitoring programme. This will usually be undertaken at a larger number of points than will be used in the ongoing programme.[2]
Biochemical Oxygen Demand (BOD)	A measure of the polluting potential of (usually) aqueous wastes through the take up of oxygen by bacteria breaking down the biodegradable matter present in waters over a set period (usually 5 days).[2]
Bivalve mollusc	Means filter-feeding lamellibranch molluscs, and by extension, echinoderms, tunicates and marine gastropods.[1,3]
Centroid	The visual centre of a polygon.[2]
Classification of bivalve mollusc harvesting areas	Assignment of harvesting areas to different classes based on an official monitoring programme to determine the extent of microbiological contamination in production and relaying areas. The requirements are given in Annex II, Chapter II of Regulation (EC) No 854/2004.[2]
Coliform	Gram negative, facultatively anaerobic rod-shaped bacteria which ferment lactose to produce acid and gas at 37°C. Members of this group normally inhabit the intestine of warm-blooded animals but may also be found in the environment (e.g. on plant material and soil).[2]
Combined Sewer Overflow (CSO)	A system for allowing the discharge of sewage (usually dilute crude) from a sewer system following heavy rainfall. This diverts high flows away from the sewers or treatment works further down the sewerage system and thus avoids overloading of works and flooding of properties, etc.[2]
Competent authority	Means the central authority of a Member State competent for the organisation of official controls or any other authority to which that competence has been conferred; it shall also include, where appropriate, the corresponding authority of a third country.[1]
Dry Weather Flow (DWF)	The daily rate of flow of sewage (including domestic and trade), together with infiltration, if any, in the sewer during dry weather. This may be measured after a period of 7 consecutive days during which the rainfall has not exceeded 0.25 mm.[2]
Emergency Overflow (EO)	A system for allowing the discharge of sewage (usually crude) from a sewer system or sewage treatment works in the case of equipment

[1] Definition from EU legislation.
[2] Supplementary definition.
[3] The requirements of the legislation for bivalve molluscs other than depuration, also apply to echinoderms, tunicates and marine gastropods. Non filter feeding gastropods are excluded from provisions on the classification of production areas.

	failure.[2]
Enteric viruses	A group of unrelated viruses that have the common characteristic of being transmitted via the faecal-oral route. The group includes norovirus and hepatitis A virus.[2]
Escherichia coli (*E. coli*)	Faecal coliform which also forms indole from tryptophan at 44°C ± 0.2°C within 24 hours.[1,4]
Established classification	Classification determined on the basis of time-series monitoring data intended to reflect annual and seasonal variation (see also Primary established classification). [2]
Faecal coliforms	Facultative aerobic, gram-negative, non-sporeforming, cytochrome oxidase negative, rod-shaped bacteria that are able to ferment lactose with gas production in the presence of bile salts, or other surface active agents with similar growth-inhibiting properties, at 44°C + 0.2°C within 24 hours.[1,5]
Flesh and intravalvular liquid (FIL)	The muscles and organs of a bivalve mollusc together with the liquid contained within the shells when the animal is tightly closed out of the water.[2]
Established classification	An official classification based on results from an extensive number of sampling occasions to ensure that potential seasonal and annual variability has been fully covered. [2]
Geographical Information System (GIS)	A computer based system that combines mapping and data storage functions in order to store, manipulate, analyze, display and interpret spatially referenced data.[2]
Geometric Mean	The geometric mean of a series of N numbers is the Nth root of the product of those numbers. It is more usually calculated by obtaining the mean of the logarithms of the numbers and then taking the antilog of that mean (see Annex 3). It is often used to describe the typical values of a skewed data set such as one following a log-normal distribution (see below).[2]
Harvesting Area	The term Harvesting Area is used in this Guide to cover both Production and Relay Areas.[2]
Hepatitis A virus	This is a 27 nm diameter virus that contains RNA as its nucleic acid. It is transmitted by the faecal-oral route and although most infections are inapparent or result in mild feverish episodes, it can cause inflammation of the liver resulting in jaundice.[2]
Hydrodynamic models	In the context of this guide, numerical models that approximate the flow of seawater, i.e. velocities and water depths as functions of time and space. Output from these models can then be used together with a representation of diffusion processes in the water column (see Particle Transport Models below) to represent the fate and dispersion of bacteria.[2]

[4] *E. coli* is a member of the faecal coliform group. It is more specifically associated with the intestines of warmblooded animals and birds than other members of the faecal coliform group. *E. coli* is determined in the reference method on the basis of the possession of β-glucuronidase activity.

[5] Usually, but not exclusively, associated with the intestines of warm-blooded animals and birds.

Initial classification	An official classification based on results from a limited number of sampling occasions. [2]
Log-normal distribution	A log-normal distribution is one in which the logarithms of the values have a normal (bell-shaped) distribution. Environmental monitoring data for many bacteria follow a log-normal distribution.[2]
Norovirus	Noroviruses are small, 27 to 32 nm, structured RNA viruses which have been implicated as the most common cause of nonbacterial gastroenteritis outbreaks. (They were formerly called Small Round Structured Viruses (SRSVs) and Norwalk-like viruses (NLVs)).[2]
Official control	Means any form of control that the competent authority or the Community performs for the verification of compliance with feed and food law, animal health and animal welfare rules.[1]
Particle Transport Models	In the context of this guide, particle transport models show the diffusion (spreading) of dissolved or suspended substances in the seawater. These methods may be used to model bacterial concentrations.[2]
Primary established classification	The first established classification determined by the competent authority after the commencement of monitoring and initial classification. Based on at least one year's monitoring undertaken at the recommended frequency. [2]
Production area	Any sea, estuarine or lagoon area, containing either natural beds of bivalve molluscs or sites used for the cultivation of bivalve molluscs, and from which live bivalve molluscs are taken.[1]
Relay area	Any sea, estuarine or lagoon area with boundaries clearly marked and indicated by buoys, posts or any other fixed means, and used exclusively for the natural purification of live bivalve molluscs.[1]
Representative sampling point	A specified geographical location from which samples are taken to represent either a single, or several, wild bivalve mollusc beds or aquaculture sites. The representative sampling point should reflect the location at highest risk of faecal pollution within the classified area.[2]
Remote area	An area where no human or animal sources had been shown to impact on the fishery in the sanitary survey and where no potential changes to sources have been identified during the annual review process. An offshore bivalve shellfishery (≥5 km from shore) not impacted by long sea outfalls is an example of a remote area. [2]
Sampler/sampling officer	In the context of this guide, a sampler is a person who takes samples of bivalve molluscs from a harvesting area (or harvested lot) for the purposes of official control testing under Regulation (EC) No 854/2004. A sampling officer is a sampler directly employed by the competent authority or other control body delegated responsibility for official control sampling.[2]
Sampling plan	A formal record of the intended sampling to be undertaken in a harvesting area with respect to species(s), position of sampling point(s) and frequency of sampling. The components of the sampling plan are identified following the sanitary survey.[2]
Sanitary survey	An evaluation of the sources of faecal contamination in or near a harvesting area together with an assessment of the potential impact of

	these sources on the microbial status of the harvesting area.[2]
Sewage	A liquid that is or has been in a sewer. It usually consists of waterborne waste from domestic, trade and industrial sources together with rainfall from subsoil and surface water.[2]
Sewage Treatment Works (STW)	Facility for treating the wastewater from domestic and trade premises. Also known as a Wastewater Treatment Plant (WWTP).[2]
Sewer	A pipe for the transport of sewage.[2]
Sewerage	A system of connected sewers, often incorporating intermediate pumping stations.[2]
Shoreline survey	A physical survey of the shoreline and area adjacent to the shore to confirm the presence of potentially contaminating sources identified through a desk-based study and to identify additional potential sources of contamination.[2]
Short-term controls	Control measures taken to reduce or negate any increased risk to public health that might arise from temporary increased contamination of harvesting areas. These controls include prohibition of harvesting, short-term reclassification and increased treatment requirement with reclassification, if necessary. The extent and period of the control measures should address the risk from the microbial pathogens, or other contaminants of public health concern, and not simply the bacterial indicators used for monitoring purposes.[2]
Storm Tanks	A tank provided to store sewage in excess of the capacity of a sewage treatment works, sewage pump or sewer capacity in the event of rainfall.[2]
Water course	A natural or artificial channel through which water flows: the term includes rivers, creeks, streams and canals.[2]

Microbiological Monitoring of Bivalve Mollusc Harvesting Areas Guide to Good Practice

1. General introduction

Consumption of raw or insufficiently cooked bivalve molluscs can result in illness due to the presence of pathogenic microorganisms. In the past, the most important illnesses associated with bivalves were typhoid and paratyphoid fevers but, with reduced frequency of these in the community, and the application of public health control measures for shellfisheries, these are now rare in developed countries such as those of the European Union. Bivalve mollusc-associated gastro-enteritis due to non-typhoid, non-paratyphoid *Salmonella* bacteria does occur from time to time but the available evidence is that this is often associated with molluscs that have not met the full requirements of the public health controls. Illnesses due to viruses, such as norovirus (causing gastro-enteritis) and Hepatitis A (causing infectious hepatitis) still occur in Europe despite the application of such controls.

An evaluation of the sources and types of faecal contamination (human and animal) in the vicinity of production and relay areas (a sanitary survey), provides the basis for determining the designated boundaries of those areas and the sampling plan for ongoing microbiological monitoring. The monitoring, based on the use of indicator organisms (*Escherichia coli* in the EU), provides an assessment of the risk of contamination with bacterial and viral pathogens. A classification is given to an area as a result of the assessment and this determines whether the areas can be used for harvesting and what level of post-harvesting treatment is needed to reduce the risk to a level that is regarded as acceptable. Ongoing monitoring determines whether the level of risk has changed and thus whether short-term controls need to be applied or the classification status changed. This Guide relates to the official monitoring undertaken for these purposes. It should be noted that the rate of uptake and removal of indicator bacteria (such as *E. coli*) by bivalve molluscs differs from that of many pathogens that may be present, especially viral pathogens, and therefore single or small numbers of *E. coli* results will not give an indication of the general risk of contamination by the pathogens. This means that a positive release system based on *E. coli* testing of a harvested batch is not appropriate and the testing of batches on receipt at a purification or dispatch centre only provides an additional check on microbiological quality and does not replace the requirement for a proper monitoring and classification system.

In the EU, the responsibility for developing and applying official monitoring programmes lies with the competent authority and the monitoring requirements are given in Annex II of Regulation (EC) No 854/2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. Associated requirements for the industry are given in Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin. The criteria given for classification in Regulations (EC) No 854/2004 and (EC) No 1021/2008 and, by cross-reference, in the Council Regulation on microbiological criteria for foodstuffs, are shown in Table 1.1.

The application of monitoring programmes has tended to vary significantly between Member States and meetings of the reference laboratory network for the monitoring of bacteriological and viral contamination of bivalve molluscs agreed that a good practice guide should be developed in order to provide a common baseline for the protection of public health and promotion of intra-community trade.

Table 1.1 Criteria for the classification of bivalve mollusc harvesting areas

Class	Microbiological standard[1]	Post-harvest treatment required
A	Live bivalve molluscs from these areas must not exceed 230 MPN *E. coli* per 100 g of flesh and intra-valvular liquid[2]	None
B	Live bivalve molluscs from these areas must not exceed, in 90 % of the samples, 4 600 MPN *E. coli* per 100 g of flesh and intravalvular liquid. In the remaining 10 % of samples, live bivalve molluscs must not exceed 46 000 MPN *E. coli* per 100 g of flesh and intravalvular liquid.[3]	Purification, relaying or cooking by an approved method
C	Live bivalve molluscs from these areas must not exceed 46 000 *E . coli* MPN per 100 g of flesh and intravalvular liquid[4]	Relaying or cooking by an approved method
Prohibited	>46 000 *E. coli* MPN per 100 g of flesh and intravalvular fluid[5]	Harvesting not permitted

Notes: [1]The reference method is given as ISO 16649-3.

[2]By cross-reference from Regulation (EC) No 854/2004, via Regulation (EC) No 853/2004, to Regulation (EC) No 2073/2005.

[3]From Regulation (EC) No 1021/2008.

[4]From Regulation (EC) No 854/2004.

[5]This level is not specifically given in the Regulation but does not comply with classes A, B or C. The competent authority has the power to prohibit any production and harvesting of bivalve molluscs in areas considered unsuitable for health reasons.

Within the Guide, aspects that derive directly from EU regulations are identified as “Requirements”. “Recommendations” have been produced by the Working Group to conform to more general wording in the regulations, or to identify good practice in the application of monitoring programmes in order to meet the requirements or intent of the regulations. For either of these, additional detail may be given under a heading “Recommended Approach”. For each Requirement or Recommendation, an “Explanation” is normally given in order to explain the public health or scientific rationale.

The guide is based on available scientific knowledge and experience gained from operating practical monitoring programmes. The guide, originally produced in 2006, was thoroughly reviewed in 2009/10 to take into account changes in legislation and experience gained in its application. The present revision incorporates some further editorial changes noted during the use of Issue 4, amendments to terminology to be consistent with the Community Guide, and removal of specific criteria for stability that were previously given in Section 3.11, further to a decision taken at the 2012 workshop of National Reference Laboratories. To assist in its application, and to yield a sound basis for further reviews, it would be beneficial for a programme of applied research to be undertaken with respect to the bacterial indicator (*E. coli*) and relevant pathogens (e.g. Norovirus, Hepatitis A virus and *Salmonella* spp.) in key elements of monitoring programme design, e.g. variation between bivalve species, spatial and temporal variability, sampling and sample transport effects.

2. Sanitary surveys

2.1 Introduction

Table 2.1 shows the sources that may give rise to faecal contamination of bivalve mollusc harvesting areas. The sources of greatest impact will differ from area to area, depending on the relative contributions of the sources in a particular area, the compounding effect of rainfall on the contribution from the individual sources (such as effectiveness of sewage treatment processes, discharges from combined sewer and surface water overflows, river flows, farming activities, direct land run-off) and the geographical proximity of the source(s) and harvesting areas. The way that tides and currents take the contamination from the source to those areas, and the effect of other environmental factors such as season, temperature, sunshine and wind, will alter the magnitude of the contamination arising from any one source. Tourism may have the effect of increasing the loading to sewerage and sewage treatment systems, and increase the number of recreational boats in an area, during certain times of the year.

Table 2.1 Sources of faecal contamination of bivalve mollusc harvesting areas[1]

Source	Level of risk to public health
a. Point Source Discharges	
Private/municipal sewage plant	Most significant risk because of diverse contributing population and volume; dependent on various factors including volume of sewage, type of treatment and plant performance
Industrial waste sources (meat processing plants, etc)	Significant risk if wastes involve pathogens capable of causing human disease, or chemicals which can be bio-accumulated; important primarily because of volume of wastes
Combined sewer overflows	Significant risk because of untreated human waste contribution and volume
Septic tanks/soakaways	Low risk because of small volumes. May be significant local risk if not operating properly.
Storm drains, street runoff	Potential risk because human sewage contamination may be present; risk significantly less than with combined sewers
Farmyards/poultry houses	Potential human risk because of large aggregation of animals and ability of some domestic animal s (pigs, fowl, cattle) to transmit human diseases
b. Non-point Source Discharges	
Waste discharges from boats	Potential risk due to possible intermittent discharge of small quantities of raw sewage
Rural land with domestic animals	Significantly less risk (farms, pastures, etc) than direct human sources
Nature reserve, forest, marsh, etc (dominated by wild animals and birds)	Significantly less risk than human sources on present evidence

[1]Modified from Gareis, 1994

Regulation (EC) No 854/2004 states that if the competent authority decides in principle to classify a production or relaying area, it must:

(a) make an inventory of the sources of pollution of human or animal origin likely to be a source of contamination for the production area;
(b) examine the quantities of organic pollutants which are released during the different periods of the year, according to the seasonal variations of both human and animal populations in the catchment area, rainfall readings, waste-water treatment, etc.;
(c) determine the characteristics of the circulation of pollutants by virtue of current patterns, bathymetry and the tidal cycle in the production area;

and

(d) establish a sampling programme of bivalve molluscs in the production area which is based on the examination of established data, and with a number of samples, a geographical distribution of the sampling points and a sampling frequency which must ensure that the results of the analysis are as representative as possible for the area considered.

Parts a-c above constitute a sanitary survey.

Regulation (EC) No 854/2004, Chapter II Annex II also states that:

Sampling plans to check the microbiological quality of live bivalve molluscs must take particular account of:

(a) the likely variation in faecal contamination,

and

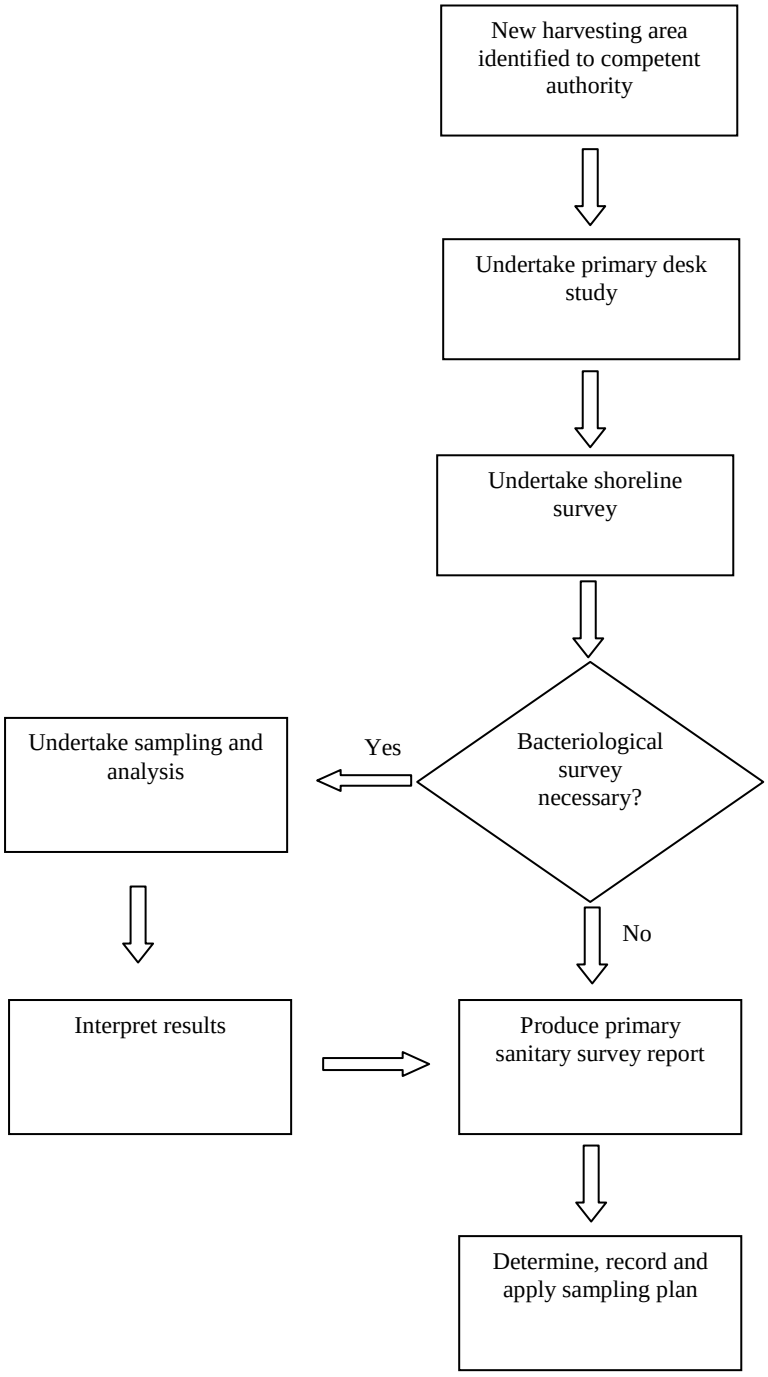
(b) the parameters referred to in paragraph 6 of Part A.

Paragraph 6 Part A of Annex II, Chapter II of Regulation (EC) No 854/2004 includes items a-d and thus the contents of the sanitary survey should influence the content of the sampling plan (see Section 3). The stages in the production of the primary sanitary survey are shown in Figure 2.1.

The Community Reference Laboratory has been informed that it is the view of the Commission that the sanitary survey requirements only apply to areas newly classified after 1 January 2006 and areas that the competent authority reclassify (this includes areas where the classification status had been either upgraded or downgraded). This has the potential to lead to two different standards of harvesting areas, one where relatively full information is available on sources of contaminants and one where potentially no information is available. The advice of the working group is therefore that, for all harvesting areas classified as at 31 December 2005, Member States should complete sanitary surveys by 1 January 2015 at the latest. This represents an extension to the recommendation given in the original version of this Guide and recognizes that development of EU-wide training and the resourcing

and implementation of sanitary survey programmes took a significant amount of time.

Figure 2.1 Sanitary survey – primary sanitary survey and production of sampling plan



2.2 Characterisation of fishery(ies)

Recommendation: The following characteristics should be identified for the bivalve mollusc fishery(ies) in a harvesting area:

a) Location and extent
b) Bivalve species
c) Aquaculture or wild stocks
d) Growing method: e.g. bottom, trestle, rope, bouchot.
e) Capacity of area
f) Production area or relay area
g) Seasonality of harvest
h) Harvesting techniques
i) Any controls under other legislation (e.g. closed seasons for the purpose of bivalve conservation)

Explanation: Knowledge of the characteristics of the fisheries is necessary for the proper interpretation of the potential effects of contaminating sources and any subsequent decisions relating to seasonal classifications or applicability of short-term control measures.

2.3 Identification of pollution sources

Requirement: Make an inventory of the sources of pollution of human or animal origin likely to be a source of contamination for the production area; and examine the quantities of organic pollutants which are released during the different periods of the year, according to the seasonal variations of both human and animal populations in the catchment area, rainfall readings, waste-water treatment, etc.;

Explanation: Faeces from both humans and animals can be a source of pathogens that may be transmitted to man via contaminated bivalve molluscs. Although human faeces may be seen as presenting a higher risk, several pathogens that infect humans can be present in animal faeces and there is presently insufficient evidence to consider risk from the two sources differently.

Recommended approach: As much information as possible should be obtained from existing sources of information and other government bodies in order to minimize the resources needed. For example, information may be available from characterization reports and pollution reduction plans undertaken for the purposes of the new Bathing Waters Directive (European Communities, 2006a) and the Shellfish Waters Directive (European Communities, 2006b). The information to be obtained and recorded should primarily, but not exclusively, cover:

1) Continuous sewage discharges
    a) Location (Latitude/longitude and/or relevant national grid reference (NGR))
    b) Size (dry weather flow, maximum flow; population equivalent if other information not available) (cubic metres per day)
    c) Treatment level (e.g. untreated, primary, secondary, tertiary, disinfected, septic tank, soakaway)

d) Tidal phasing or other periodicity if relevant

2) Rainfall-dependent sewage discharges (combined sewer overflows or storm tank overflow) and other rainfall-dependent discharges (stormwater discharges)

    a) Location (Latitude/longitude and/or relevant NGR)
    b) Measured or predicted spill frequency (per annum)
    c) Treatment level (if any)
    d) Tidal phasing or other periodicity if relevant
    e) Maximum flow rate (litres per second)

3) Emergency discharges
    a) Location (Latitude/longitude and/or relevant NGR)
    b) Circumstances under which the discharge may operate
    c) Maximum predicted flow rate (litres per second)

For the three types of discharge covered in 1, 2 and 3 above, information on the following aspects may assist in the assessment progress but it is recognized that these details may not be available to those undertaking the sanitary survey:

a) Microbial content of the associated continuous flow (results of any monitoring undertaken on the discharge together with information on the flow conditions pertaining)
b) Sanitary content of the associated continuous flow (as surrogate if microbial content not available) such as measured levels of ammonia, biochemical oxygen demand (BOD), suspended solids together with information on the flow conditions
c) Seasonal variations in any of the above

Trade discharges that have a significant sewage content should be assessed as for a continuous sewage discharge but on the basis of the proportion of flow that is sewage or other source of faecal contamination (slaughterhouse content, etc). The effects of any antimicrobial action of the chemical constituents should also be estimated.

4) Land use
    The following is a guide to type of land-use that may be recorded:
    Pasture land
        Cattle
        Sheep
        Pigs
        Horses
        Poultry
        Other livestock
    Arable
    Grassland
    Horticulture
    Forest/Woodland

Urban areas, roads and other impermeable cover

Information on seasonal variations in use and application of manure and/or sewage sludge including method of application and seasonal variations.

5) Farm animals

In relation to pasture land, penned areas and animal sheds, the location and number of animals should be recorded, with any seasonal variations, as well as the location and management regime for any slurry pits, etc.

6) Wildlife

Information on significant (large number in general vicinity; smaller number close to the bivalve mollusc fishery) populations of wild animals and birds.

Record: type of wildlife; location (as accurately as possible); approximate numbers; seasonal variations

7) Ships and Boats

Record the presence of harbours, marinas and mooring areas with numbers of boats (split into general categories) and the number of persons who may be living on board. Record whether there are restrictions on the discharge of waste and whether (practical) pump-out facilities are provided. Areas where relatively large numbers of boats pass through should also be noted.

2.4 Storage of data

Data will preferably be stored in database form capable of being linked to a geographic information system (GIS) for display purposes. Database guidelines for microbiological data are given in Section 6 and these may be applied *mutatis mutandis* to the data from sanitary surveys. Display of items on a map, preferably within a GIS, facilitates interpretation of the information, especially when a number of different data sets are involved (e.g. location and type of fishery, location and nature of polluting sources, existing sampling points).

2.5 Validation of data

Large-scale validation of data may not be feasible. However, any provisos regarding the validity of the data should be sought from the organization providing the data and at least simple validation procedures should be undertaken (e.g. are all relevant fields completed; does displaying the location in a GIS show that objects that should be on the land or in the water plot as expected?).

2.6 Shoreline surveys

Recommendation: Shoreline surveys should be undertaken in order to determine whether all significant sources of contamination have been revealed by the desk-based study and whether previously identified sources are still present.

Explanation: A shoreline survey is a physical inspection of the shoreline and area in the vicinity of a harvesting area in order to confirm that the potential sources of contamination identified by the desk study are still extant and to identify any additional potential sources not revealed by the desk study.

Recommended approach: The whole shoreline in the vicinity of the bivalve mollusc fishery should be subject to a survey. As part of the desk study, an assessment needs to be made as to the extent that the survey needs to extend beyond the immediate vicinity of the fishery (e.g. upstream). The aim is to confirm the information on the location and extent of the bivalve mollusc fishery and presence of sources of contamination identified within the desk study, and to identify additional sources of contamination that might impact on the fishery. As much information as possible should be noted on the types of contaminating sources given in the section above on the desk-based survey. Where possible, samples should be taken from any previously unidentified discharges operating at the time of the survey and from any watercourses discharging near harvesting areas, and bivalve molluscs nearest to these sources. It should be noted that not all potential contaminating sources will necessarily be identified during a single survey, e.g. there may be seasonal differences in the presence of some factors (tourism, number and location of animals) while in dry weather, land drains and other rainfall-dependent sources may not be operating. Additional surveys may therefore be necessary in response to unexplained high results in the monitoring programme and such surveys may need to be timed to coincide with factors thought likely to lead to higher levels of contamination. Items that may be needed during the survey include means of determining and recording the location and nature of observations (maps, (GPS, camera), materials for taking and transporting samples, and equipment deemed necessary from a health and safety perspective (including tidal information).

Undertaking shoreline surveys can be hazardous and appropriate risk assessments should be prepared and followed.

The following types of information should be recorded:

Name(s) of surveyor(s)
Date Start and end times
Name of surveyed harvesting area
Extent of surveyed area (from….to….)
Tidal state at time of survey
Weather (precipitation over last 48 hours; cloud cover, precipitation, wind direction, wind speed at time of survey)
Location and extent of bivalve mollusc beds

- clarification of location of routine bivalve mollusc sampling points
- clarification of location of routine water microbiology sampling points – shellfish waters, bathing/recreational waters, river quality

Confirmation of location and nature of sewage and other discharges identified during the desk-based survey
Identification of location and nature of sewage and other discharges not identified during the desk-based survey
Identification of waterways (rivers, streams) discharging near to the harvesting area

Record of type of use of land adjacent to the shore (e.g. forest, grassland, pasture, arable, horticulture, urban)
Presence, approximate number and location of farm animals on land adjacent to the shore
Presence, approximate number and location of other animals or birds on land adjacent to the shore
Presence, approximate number and location of animals or birds in/on the water
Location and number of moored or other ships and boats together with a note as to whether there are specific local controls on discharges from these and whether pump-out facilities are provided in harbours or marinas
Other relevant observations, e.g. presence of algal blooms, sediment dredging operations, etc.

An example record form is given at Annex 1.

Samples of shellfish and water should be taken as determined at the time of the survey. Water samples may include: watercourses, previously unidentified discharges of unknown origin or type, other discharges if the microbial content is not known from other sources, seawater in the vicinity of the bivalve mollusc fishery.

Photographs are often useful in placing the records of the survey in context and for providing additional information not recorded at the time of the survey.

2.7 Hydrography/hydrodynamics

Requirement: Determine the characteristics of the circulation of pollutants by virtue of current patterns, bathymetry and the tidal cycle in the production area;

Explanation: The depth of water and currents in an area will affect the extent of dilution of contaminants and also the way that these contaminants will impact on nearby bivalve mollusc fisheries. This will markedly influence the level of microbiological contamination of the bivalves and, with regard to currents, how this varies with time (due to tidal and wind effects, etc). Knowledge of these effects is therefore important in interpreting the information on sources of pollutants obtained for the sanitary survey.

Recommended approach: For hydrography, nautical charts should be available for the area either within a GIS (the preferred approach) or as hard copies. For hydrodynamics, there are three levels of approach:

1) Tidal charts/tidal stream software.
   This is the minimum level that can be judged to meet the requirements of the legislation. They can be used to roughly estimate the direction and distance traveled of contamination from major sources. Appropriate information may not be available for many areas such as small to medium size estuaries, rias or sea lochs.

2) Simple hydrodynamic modeling.

Generic software packages are available that enable simple two-dimensional modeling of the effects of contamination sources given data on depths and current flows. The hydrodynamic model for an area is best set up and validated by a specialist modeler. A particle-tracking model can then be used by other technical staff to investigate the fate of contaminants from a point source discharge. It may be possible to represent some non-point source discharges as point sources in such models, e.g. a river may be represented as a point source at the tidal limit. The use of these models requires depth and boundary state tidal information and this may not be available for all harvesting areas. Many of these models will not cope well with narrow estuaries, sea lochs with limited tidal exchange or large areas that dry out at low tide. However, these models will provide information over and above that given by tidal charts/tidal stream software and should be considered for harvesting areas:

a) with large production;
b) where the sanitary survey and any bacteriological surveys give conflicting results;
c) where unexpected high *E. coli* results are obtained relatively often in the routine monitoring programme;
d) associated with a number of suspected outbreaks.

3) Complex hydrodynamic modeling.
Such two or three-dimensional models require considerable resource to set up and validate. They will usually perform much better than the simple models but will usually be too expensive and time consuming to consider only from a bivalve mollusc fishery perspective. Output from such models may be available from investigations undertaken for other purposes, e.g. large sewage improvement schemes. Where available, these should be used in the sanitary survey process.

The effects of wind and density drive currents may be significant and should be taken into account where possible.

Alternative or complementary approaches are the use of dilution estimation (which may include output from dye dosing or salinity studies) and tracing using chemicals (e.g. rhodamine WT or fluorescein) or microbes (e.g. phages of *Enterobacter* or *Serratia* or *Bacillus globigii* spores). Where possible, deliberate introduction of substances into the vicinity of a fishery should be undertaken outside of periods of active harvest.

2.8 Analysis of historical microbiological data

Recommendation: Where historical microbiological monitoring data is available from shellfish hygiene (Regulation (EC) No 854/2004), shellfish waters (under the Water Framework Directive, 2000/60/EC) or bathing waters (Directive 2006/7/EC) monitoring programmes for the immediate, or nearby, area, this should be analysed to determine whether it will inform the overall assessment and recommendations of the sanitary survey. Where available, analysis of such data should supplement and not override the other elements of the sanitary survey.

Explanation: Historical monitoring data for faecal indicator bacteria will give actual information on geographical and temporal (including seasonal) variation in the extent to which sources of faecal contamination impact on the water and/or bivalve mollusc quality in an area. Spatial (geographical) variation is probably of most direct relevance to the sampling plan. If sufficient data is available, statistical analyses may also yield information on the effect of environmental factors (such as tide, rainfall and wind). However, the relevance of the data should be critically assessed with respect to sampling location relative to the bivalve shellfishery which is the subject of the sanitary survey, the time period of the available data and, where appropriate, the bivalve species for which the data is available. Presentation of data which is not relevant will confuse, rather than inform, the overall sanitary survey assessment. In general, unless the outcomes will assist in other aspects of the monitoring programme, analyses should be limited to those that will inform the sanitary survey outputs.

2.9 Bacteriological surveys

Recommendation: If the best location for one or more representative sampling points for an area is not clear after doing the desk study and shoreline survey, it is recommended that a bacteriological survey is undertaken to clarify the location and extent of contamination. Several potential points should be identified from the results of the desk study and shoreline survey. It is then recommended that at least 3 samples are taken from each site at intervals not closer together than fortnightly and tested for *E. coli*. Taking seawater and/or surface sediment samples as well as bivalve mollusc samples may provide additional information. Depending on the outcome of the desk-study and shoreline survey, the bacteriological survey may be targeted towards conditions that are considered to increase the risk of contamination of bivalve molluscs in the specific area (e.g. rainfall, specific tidal conditions).

The geometric means, minima and maxima of results at each sampling point should be calculated and recorded along with the raw data. The sampling point or points showing the highest maximum *E. coli* concentrations should be selected for the monitoring programme. Where the maximum concentrations are similar, the site or sites showing the highest geometric mean *E. coli* concentration should be selected.

Explanation: Qualitative or quantitative assessment of the effects of contaminating sources is complicated due to the large number of factors that may modify the impact. Even after undertaking a sanitary survey, it may not be clear where representative sampling points should be located. A time-limited bacteriological survey at several potential points may provide such information. Samples need to be taken on a number of different occasions to reflect differing environmental conditions (e.g. spring/neap tidal cycles, periods of wet/dry weather, etc.). Such a limited survey will not show the effects of seasonal differences in the extent of contamination.

2.10 Salinity monitoring

Recommendation: Salinity monitoring may be undertaken for areas potential impacted by rainfall-associated discharges (sewage or surface waters) or diffuse inputs by sampling and subsequent testing (with refractometer, hydrometer or

conductivity meter) or by using a continuous monitoring apparatus. Such monitoring may be undertaken in conjunction with a shoreline survey, a bacteriological survey, or as part of the ongoing monitoring programme. It may be relevant to take salinity readings at different depths or states of tide.

Explanation: Salinity readings may give useful information on the degree to which fresh-water associated inputs impact on a harvesting area. The data does not contribute to the analysis of the microbiological data for the purposes of classification but may be used in the interpretation of other information as part of the sanitary survey report (and subsequent reviews) and may also contribute to information on the management of potential impacting sources for water quality purposes.

2.11 Compilation of the sanitary survey report

A report of the information and assessment should be prepared. This should include the following:

- Overview of bivalve mollusc fishery
- Fishery
  - Location and extent
  - Bivalve species
  - Aquaculture or wild stocks
  - Production area or relay area
  - Seasonality of harvest
  - Harvesting techniques
  - Any controls under other legislation
- Location, size and treatment level of human sources of contamination
- Location and estimated volume/load of agricultural sources of contamination
- Significant wild animal/bird populations
- Maps, seasonality effects, for these factors
- Records of shoreline surveys
- Hydrography/hydrodynamics
- Analyses of historical microbiological data
- Records of bacteriological survey results
- Assessment of effect on contamination of bivalve molluscs

The report should contain maps of the relevant information in order to help interpretation.

2.12 Assessment of sanitary survey data

Recommendations: There are potentially three different levels to the assessment of the data once it has been assembled.

Qualitative assessment For each potential source, an assessment should be made as to whether it will contribute to the microbial load at the bivalve mollusc fishery. This assessment will initially need to consider the microbial load of the source, its interaction with other sources, the distance from the fishery and the dilution of the

source material in the water. Assuming that this indicates that an impact could occur, a subsequent hydrodynamic assessment should be made to see whether there is still an impact when currents are taken into account (this will need to take into account the effect of spring/neap as well as high/low tidal cycles, and the possible influence of wind and thermo- or haloclines. Seasonal effects may also be relevant).

Where the contribution from a source cannot be discounted on the basis of a qualitative assessment, but where the significance of the impact is uncertain, a semi-quantitative or quantitative assessment may be justified.

Semi-quantitative assessment This is the first level of further assessment that may be considered if a qualitative assessment has proven inadequate. It may also be considered as a default level of assessment if the data and resources allow. The first stage is to assign a risk ranking to the various sources identified during the qualitative assessment process. This ranking will usually be undertaken in terms of both the loading of the source (in terms of *E. coli*) and the distance from the shellfishery (or part of shellfishery). The second stage is to identify the combined risks at different relevant locations in the harvesting area (e.g. different bivalve mollusc farms or beds; different locations on a large farm or bed). This will result in a relative assessment of the risk of contamination from all significant sources at the different locations.

Quantitative assessment This will normally necessitate the use of a particle-tracking model in conjunction with a hydrodynamic model and estimation of the microbial load in the water at the fishery. Additional modelling may be used to convert the predicted concentrations in the water column into concentrations in the bivalve molluscs.

In each phase, particular attention needs to be given to circumstances where intermittent sources of contamination may not be adequately reflected by a regular monitoring programme, especially if constraint on tidal exchange may potentiate the effects. Special consideration also needs to be given to any circumstances whereby the monitoring programme based on *E. coli* may not adequately reflect the pathogen risk (e.g. a major discharge disinfected by chlorination). In such cases, it should be considered as to whether the area will need to be classified at a worse level (e.g. C rather than B) than the monitoring data would suggest, or whether harvesting should be allowed at all.

Explanation: A qualitative assessment may be sufficient in many cases to determine whether or not each particular source is likely to impact on the microbial status of the fishery. This approach should be taken before any semi-quantitative or quantitative assessment is considered. The primary intent of the sanitary survey is to ensure that the sampling plan, with regard to number of sampling points and sampling frequency, adequately reflects the likely sources of contamination in the resulting data set(s) used for classification and that the resulting classifications properly reflect the likely risk of pathogen contamination. Where the sanitary survey identifies that this risk cannot be reflected by a practical sampling plan this should be made clear to the competent authority so that the consequences can be assessed

and the appropriate action taken in order to protect public health. Particular examples are addressed in more detail in Section 2.13.

The survival characteristics and persistence in seawater and shellfish of the bacterial indicator (*E. coli*) used in the monitoring programme, and of the pathogens of importance in bivalve-mollusc associated need to be considered in assessing the sanitary survey data.

2.13 Closure areas around outfalls, harbours and marinas[6]

Recommendation: Areas within active harbours and marinas should not be used for the harvesting of bivalve molluscs. Areas within presently inactive harbours and marinas (including those used on a seasonal basis) should not be used for harvesting unless a study of both microbiological and chemical contamination has shown that this is below a level that could cause a risk to human health from consumption of the bivalves. Class A zones should not include continuous or intermittent sewage or animal slurry discharges or the mixing zones of these. Class A zones should also not include a zone of 300 metres radius around the entrances to harbours or marinas or any other outflows from these, unless the sanitary survey shows that this exclusion zone can be reduced. Such exclusions should also be considered for Class B zones unless a tracer exercise or water quality modeling study, combined with bacteriological monitoring in the immediate vicinity of the source, has shown that there is no potential impact. Chemical contaminants may be a particular problem with some outfalls containing industrial wastes and with harbours and marinas and the potential effects of these will need to be assessed separately.

Explanation: While the primary objective of the sanitary survey under Regulation (EC) No 854/2004 is to inform the sampling plan to be established for an area, the exercise will identify sources that will contribute directly to the microbiological contamination of the area and will constitute an unacceptable risk to public health if bivalve molluscs are harvested from their vicinity. As identified in Sections 2.11 and 2.12, practical sampling plans may not be able to yield results that adequately reflect the risk of contamination by pathogens and therefore relevant data obtained during the sanitary survey, as well as the results of any monitoring, need to be taken into account when determining the appropriate controls to be applied.

2.14 Review of sanitary survey

If regular information on changes to the potentially contaminating sources in an area is received from the responsible bodies then that information should be reviewed as it is received, in conjunction with the other information available since the sanitary survey (or last review) was undertaken and a decision taken as to whether a formal review of the sanitary survey is necessary or whether the sampling plan needs to be revised. Otherwise an annual review should take place to ensure that the environmental conditions have not changed and that the classifications are still valid. This process includes:

[6] See Annex 4 for additional criteria relating to areas to be approved for export of live bivalve molluscs to the United States of America.

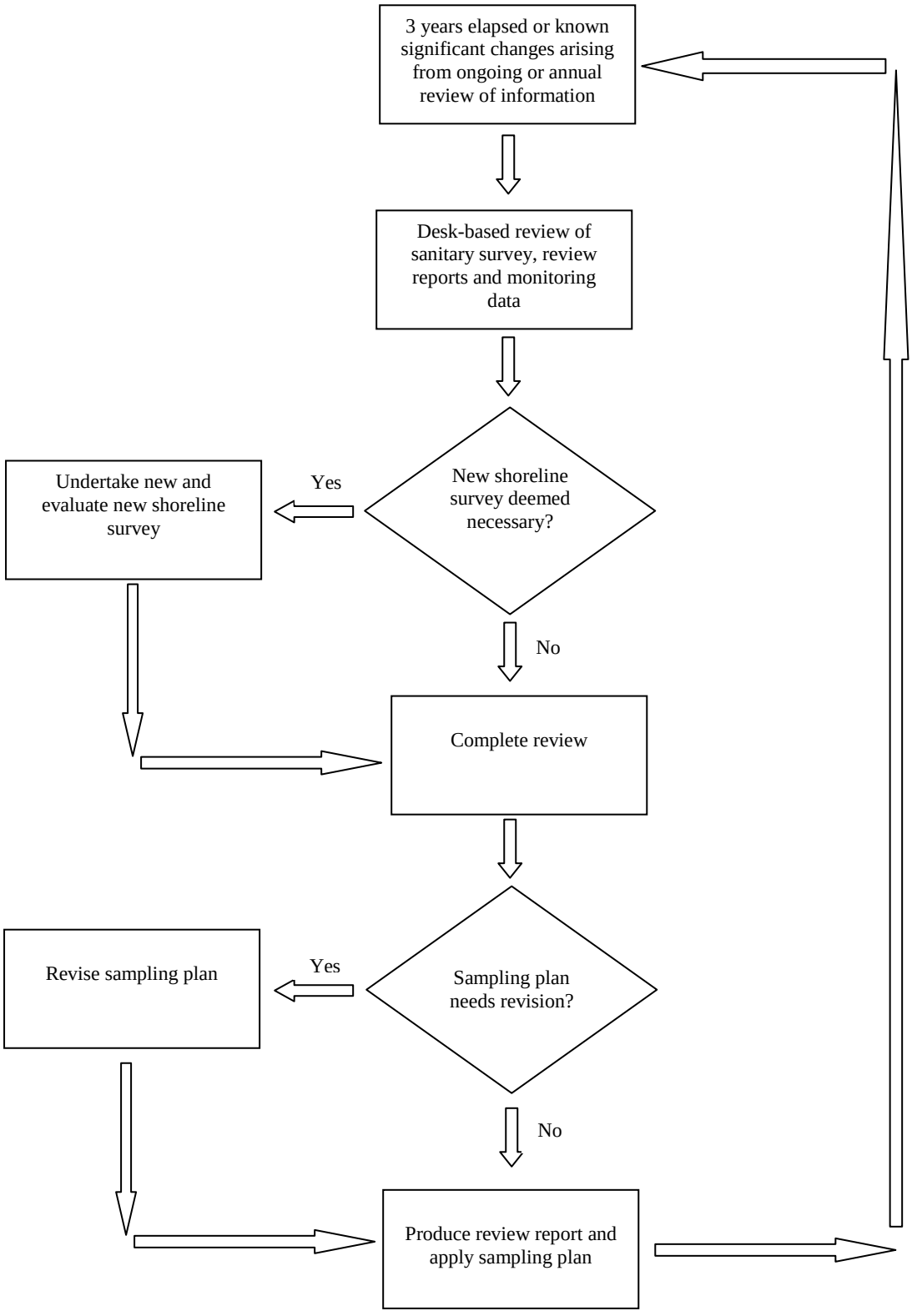
a) file review on the status of all bivalve mollusc growing areas (including routine microbiological monitoring);
b) performance records for all sewage treatment works and industrial discharges;
c) a status report on abatement of pollution from sources identified during past sanitary surveys;
d) evaluation of new pollution sources; and
e) bacteriological sampling at representative sampling points at a suitable frequency, if deemed necessary from the results of items a) to d).

A complete re-evaluation of pollution sources and the sampling plan should be undertaken once every six years. This may be undertaken less frequently for remote areas where no potential changes had been identified during the annual review process. The stages in the review of the sanitary survey are shown in Figure 2.2.

2.15 Outcome of the sanitary survey – the sampling plan

Regulation 854/2004 stipulates that sampling plans must be drawn up for the microbiological monitoring of relaying and production areas and that these should take account of the outcome of the sanitary surveys (see Section 3 for more details). The intent of the sanitary survey is to inform the siting of sampling points, and the timing of sampling with respect to both the time of year, for commercial fisheries that are seasonally active, and time relative to potentially contaminating influences, such as tidal effects, rainfall, etc, in order that the microbiological results that are obtained are representative of the area. Establishment of the sampling plan will need to consider the extent of the classified zone(s) (see Section 7.3 Delineation of classified zones) so that the sampling plan is appropriate to the zone(s).

Figure 2.2 Review of sanitary survey and sampling plan



3. Sampling plans - bivalve mollusc species, spatial and temporal considerations

3.1 Introduction

The results obtained in a microbiological monitoring programme will depend on the design and implementation of the programme and, in statutory programmes, this will have a direct effect on the compliance determined using the results – in terms of bivalve molluscs, this will affect the classification status of harvesting areas. The five principal factors shown to affect results are the species sampled, the location of sampling points (primarily in relation to sources of contamination), the frequency of sampling, timing of sampling (largely in relation to environmental variables) and the way that the data is assessed (period of time, tolerance allowed).

The sampling plan constitutes a formal record of the intended sampling to be undertaken in a harvesting area with respect to species(s), position of sampling point(s) and frequency of sampling. The components of the sampling plan are identified following the sanitary survey. A number of other items of information, e.g. the responsible authority and the designated sampler(s) also need to be recorded in order to ensure that the sampling plan is put into effect.

The resulting sampling plans are necessarily a compromise between the scientific assessment of the requirements necessary to properly reflect the level of microbiological contamination in a harvesting area (with a view to protecting public health) and the practicalities of obtaining, transporting and analysing the samples together with the associated costs. This compromise has to be taken into account when interpreting the resulting data (see Section 7).

Requirements:

Regulation (EC) No 854/2004 Annex II, Chapter II, A, 6:

> If the competent authority decides in principle to classify a production or relaying area, it must:
>
> d) establish a sampling programme of bivalve molluscs in the production area which is based on the examination of established data, and with a number of samples, a geographical distribution of the sampling points and a sampling frequency which must ensure that the results of the analysis are as representative as possible for the area considered.

Regulation (EC) No 854/2004 Annex II, Chapter II, B, 1:

> Classified relaying and production areas must be periodically monitored to check:
>
> (b) the microbiological quality of live bivalve molluscs in relation to the production and relaying areas;

Regulation (EC) No 854/2004 Annex II, Chapter II, B, 2:

> To implement paragraph 1(b), (c) and (d), sampling plans must be drawn up providing for such checks to take place at regular intervals, or on a case-by-case basis if harvesting periods are irregular. The geographical distribution of the sampling points and the sampling frequency must ensure that the results of the analysis are as representative as possible for the area considered.

Regulation (EC) No 854/2004 Annex II, Chapter II, B, 3:

> Sampling plans to check the microbiological quality of live bivalve molluscs must take particular account of:
> (a) the likely variation in faecal contamination,
> and
> (b) the parameters referred to in paragraph 6 of Part A.

Explanation: The intent of the legislation is to ensure that sampling plans, and thus the resulting microbiological data, are as representative of the area being monitored as possible. The recommendations given below in the rest of Section 3 are intended to comply with these requirements in a cohesive and scientifically based structure.

3.2 Recording of sampling plans

Recommendation: For each harvesting area, the elements of the sampling plan covered in Sections 3.3 to 3.12 should be formally recorded. There should also be a record of additional information relating to sampling responsibility. The key items are:

- Production area
- Site Name
- Site Identifier
- Species
- Geographical location (grid reference and/or latitude/longitude)
- Allowed maximum distance from identified sampling point
- Depth of sampling (if relevant)
- Frequency of sampling
- Responsible authority
- Authorised sampler(s): name(s) and reference number(s)
- Other relevant information

These items are discussed in detail in the sections below. It is preferable for the sampling plan to have an associated map showing the area together with the representative sampling points.

The sampling plans should be available to the competent authority, the monitoring programme manager and the samplers. Revisions to sampling plans should be recorded and made available to these personnel. The plans may also be provided to other interested parties.

Explanation: All those involved in the microbiological monitoring programme need to be aware of the sampling plans for the part(s) of the programme in which they are involved in order that the work can be carried out properly. This can only be achieved

if the plans are formally recorded and made available to those concerned. It also provides the means by which the monitoring actually undertaken can be audited against that which was expected.

3.3 Bivalve species

Recommendation: Either

1) Separately monitor each commercially harvested species

or

2) Use one or more indicator species for the area where parallel monitoring has shown that the indicator species yields results at least as high as those of the other species it represents.

Explanation: Different bivalve species can vary markedly in the levels of *E. coli* contamination that they show when they are exposed to the same quality of water. They also differ in the time of response (uptake and removal) to specific contamination events. The default recommendation is therefore that each commercially harvested species within an area be monitored separately in order that the correct classification status is given for that species and therefore that the correct post-harvesting treatment requirements are applied. Use of the indicator species approach will reduce the number of samples that need to be taken in an area where more than one species of commercially harvested species co-exist. However, if one or more indicator species is to be used, a conservative approach must be taken in order to protect public health. This means that the indicator species must yield results at least as high as those of the other species for which it acts as an indicator. Each commercially classified species should be identified separately in the resulting classification list and not just the indicator species.

The approach identified here will need to be reviewed when the guide is subject to future revisions in order to take into account any additional information on the differential uptake of relevant pathogens and the bacterial indicator by different species of bivalve molluscs.

3.4 Selection of location and number of sampling points

Recommendation: Location of representative sampling points should be based on the outcome of the sanitary survey and should reflect the location of potential contaminating sources and the impact indicated by hydrodynamic assessment. The geographical extent of an area, its commercial production potential and the extent of homogeneity with regard to contamination and other factors should be taken into account when deciding on the number of sampling points. If an area is split into separate enforceable units, each capable of being classified at a different level (if necessary), or subject to separate short-term closures, at least one sampling point should be located in each unit.

Explanation: *E. coli* concentrations in a single bivalve mollusc species may vary markedly across a harvesting area and this variation may in itself vary from one sampling occasion to another. Sampling points need to be identified that detect this

variation. However, for the purposes of public health protection, it is important that at least one sampling point is placed in each separately enforceable area.

3.5 Geographical identification of sampling points

Recommendation: Each representative sampling point should be at a fixed geographical location, identified by latitude/longitude or national grid reference to an accuracy of 10 metres. Samples should be taken within an identified distance of this location – for hand-picked or raked samples, this should be within a maximum of 50 metres of the identified point and for dredged samples this should be within a maximum of 250 metres. These maximum values may not be appropriate in some fisheries and may need to be amended larger or smaller) as a result of the sanitary survey. The maximum allowed tolerance around the designated sampling point should be recorded in the sampling plan. If it proves difficult to obtain sufficient animals for a sample on a number of occasions, consideration should be given to identifying a new point, again based on the sanitary survey, where more reliable samples can be obtained. The old point should then be discontinued.

Explanation: The extent of contamination, as indicated by *E. coli*, will vary both spatially and temporally. It is necessary to identify fixed sampling points in order to minimise the complication of variability due to both factors occurring at once. Some latitude is needed around the points, particularly with wild stocks, as the density of the beds will vary. Dredging runs will often be undertaken over several hundred metres and the latitude for samples obtained this way is therefore greater. In order to maintain the fixed location concept, it is necessary to replace a sampling point that does not produce sufficient animals for testing with another that does. This sampling point has to be identified on the same basis as the original.

3.6 Offshore sampling points

Recommendation: Where a harvesting area lies at least 5 km from the shore (i.e. no point within the area is nearer than 5 km to the shore), and the sanitary survey (including appropriate modelling) shows that no source of faecal contamination impacts on the area, and the area is therefore homogeneous with respect to microbiological quality, a virtual sampling point may be identified at the centroid of the area instead identifying a fixed sampling point as in Section 3.5. Samples should then be collected from non-fixed points in the area on a frequency given by Sections 3.7 to 3. 11, as appropriate, and the exact positions noted at time of sampling. The classification for such harvesting areas should be assessed on the basis given in Section 7.3 as though the samples all originate from the virtual sampling point.

At each review of the sanitary survey the results should be assessed for any spatial trends in the results. If such assessment shows significant differences in the contamination across the harvesting area, the area should be subdivided and separate sampling undertaken for each subdivision.

Explanation: In the case of offshore harvesting areas it may be difficult to collect samples from a fixed sampling point on a continuous basis. Where the sanitary survey has shown that no source of faecal contamination impacts on the area, samples taken from anywhere within the area may be deemed to represent it. The samples are assigned to a virtual sampling point for ease of reference and data

analysis. Ongoing assessment for potential spatial variability is necessary to ensure that the assumptions made during the sanitary survey are correct and, if not, the area subdivided so that separate assessment can be made on each subdivision.

3.7 Depth of sampling

Recommendation: Where bivalve molluscan shellfish are grown on ropes or bouchots, samples should be taken at the depth that generally yields the highest *E. coli* results. During initial monitoring, it will therefore be necessary to take parallel samples at more than one depth so that this can be determined. Where bagged bivalve molluscs are used for sampling instead of the normal harvested stocks, the bag should be located as near in depth to those stocks as possible.

Explanation: The extent of microbiological contamination of bivalve molluscs grown on ropes or bouchots can vary markedly with depth. The effect may vary from area to area and may not be predictable. A number of factors may be involved, e.g. more contaminated fresh water floating over more cleaner, more saline water (worse results near the surface) or suspension of contaminated sediment (worse results nearer the bottom). It is therefore necessary to evaluate the effect for a specific location by taking samples at more than one depth on a number of occasions and comparing the results. The depth that generally yields the highest *E. coli* results should be used for subsequent sampling as this will be more protective of public health.

3.8 Sampling frequency – initial classification

Recommendation: For initial classification of an area, it is recommended that at least 12 samples are taken from each identified sampling point over at least a 6 month period with the interval between any two successive sampling occasions being not less than one week. If the sanitary survey shows that the area is remote with no significant sources of pollution it is recommended that at least 6 samples be taken over a period of at least 3 months with the interval between any two successive sampling occasions being not less than one week. The results of the testing of bivalve molluscs in any bacteriological survey taken at the identified sampling points can count towards this requirement as long as the recommended interval between sampling occasions is respected. Where possible, the period of the year used for monitoring towards an initial classification should be that identified during the sanitary survey as that most likely to yield the highest results.

Explanation: The likelihood of autocorrelation (positive association) in *E. coli* concentration in consecutive samples is more likely to occur the closer together samples are taken in time. Separating sampling occasions by the recommended period will reduce this likelihood while enabling time series data from the recommended number of samples to be obtained within a reasonable period. It should be noted that a six month monitoring period may be insufficient to reveal seasonal patterns and thus undertaking this procedure during the period presumed to yield the worst results will help to ensure that public health is protected during the period between initial and primary established classification.

3.9 Sampling frequency – primary established classification

Recommendation: After initial classification, areas should be monitored at least fortnightly for a year (from the start of monitoring towards a primary established classification) so that an established classification can be obtained, unless a different frequency has been recommended following a sanitary survey.

Explanation: Data for the purpose of an initial classification will usually be acquired over a period of less than a year and will be potentially subject to seasonal differences, meteorological effects, etc. It is therefore important that a relatively high frequency of monitoring is maintained for a complete year in order to properly assess the level of contamination in the area covering all seasons. It will still be the case that longer-term variations (e.g. variations in annual rainfall) will not be shown in such monitoring. However, a different frequency may be justified on the basis of the scientific assessment within a sanitary survey.

3.10 Sampling frequency – ongoing monitoring (<3 years' data)

Recommendation: The minimum sampling frequency for ongoing monitoring at sites with less than 3 years' data should be at least monthly on a year-round basis.

Explanation: Due to potential annual, seasonal and shorter-term variation in the *E. coli* results in an area, monitoring on at least a monthly basis over the first three years is necessary in order to achieve yield a proper assessment of the classification status of an area.

3.11 Sampling frequency (ongoing monitoring ≥ 3 years' data)

Recommendation: The minimum sampling frequency for ongoing monitoring at sites with ≥ 3 years' data should normally be at least monthly on a year-round basis. However, the sampling frequency may be reduced to bimonthly for areas that conform to the definition of remote (see Glossary) and where the official classification status over the previous three years has remained the same.

If a review of the sanitary survey, or results of ongoing monitoring, indicate that the extent of contamination in an area or zone identified for bimonthly sampling has changed, then at least monthly sampling should be instituted.

Explanation: The concentration of *E. coli* in bivalve molluscs at a specific sampling point will usually vary greatly over a few hours. For time series analysis of data, it is important to sample regularly and on a reasonably frequent basis. The minimum frequency generally accepted to yield a useful data set is monthly. However, some harvesting areas will yield *E. coli* results that do not fluctuate markedly between sampling occasions and where the results are clearly compliant with one class in the long term. Analyses undertaken by the EURL on a large set of *E. coli* monitoring data has not yielded any descriptive statistics that can clearly define areas as stable . This may be due to variation in both sources of faecal contamination and in the environmental factors that influence how such contamination affects *E. coli* concentrations in bivalve molluscs. It has therefore been concluded that only areas that have be demonstrated to be remote from potential sources of pollution, and for

which the classification status has stayed the same for at least three years, should be considered for a reduced monitoring frequency.

3.12 Seasonality of sampling

Recommendation: The default approach to monitoring should be that it takes place at least at the frequency identified in Section 3.11 throughout the year. Where there are clear seasonal patterns to commercial activity in class A or B areas, preferably enforced by local fishery regulations, monitoring may be considered for a reduced period of the year. This should start at least 1 month prior to the harvesting season for class A areas and two months prior to the season for class B areas and then continue throughout the season. The frequency for seasonal monitoring should be increased over that given in Section 3.11 in order to ensure that the appropriate minimum size of data set given in Section 7.3.3 is satisfied (see also section 7.3.8). Where the sanitary survey and/or historical monitoring data indicate that results >46000 *E. coli* per 100 g of F.I.L. could occur at other times, or for class C areas, monitoring should take place throughout the year. If there is a possibility that harvesting could take place outside of the traditional season for an area, then monitoring should also take place throughout the year.

Explanation: Many bivalve mollusc fisheries operate on a seasonal basis. Monitoring during the closed season may be a waste of resource which could be targeted at gaining additional data during the harvesting season. The latter is necessary in order to ensure that the minimum required data set is obtained for subsequent analysis. Increasing the monitoring frequency during a particular period of the year will increase the likelihood of detecting high results during that period.

Some pathogens, particularly viruses, may take a long time to clear from bivalve molluscs after a contamination event – this may be up to two months, depending on bivalve species and the seawater temperature. It is therefore important to monitor for a period of time before harvesting takes place. Where there is an identified risk that extreme contamination (>46000 *E. coli* per 100g of F.I.L.) could take place outside of the identified season, year-round monitoring is necessary in order to determine whether harvesting should be prohibited.

3.13 Time of sampling

Recommendation: Sampling should either be:

a) undertaken on as random a basis as possible with respect to likely influencing environmental factors e.g. tidal state, rainfall, wind etc so as to avoid introducing any bias to the results.
b) undertaken under conditions that have been identified as producing the highest levels of contamination (worst-case approach).

Recommended approach:

Randomised sampling – Ideally, sample dates within each period of time (e.g. month) should be allocated by reference to random number tables or computerized random number systems. The same approach should be taken to sampling time within each sample date (within the available time frame). Where this is not possible, sample

dates and times should be allocated without any obvious regularity in order to avoid coinciding with particular tidal states, etc. In both cases, the annual sanitary survey review should include analysis as to whether any bias towards particular states of each factor has occurred. If such a bias is detected, the sampling plan for the point should be revised to remove such bias or the worst-case approach should be taken.

Worst-case sampling – Sampling dates and times which are likely to produce the highest levels of contamination should initially be identified using the outcome of the sanitary survey. Once a significant amount of data is available for a sampling point (e.g. at least 50 results), statistical analysis may be used to reassess the effect of the various states of each factor. Due to the variability of environmental data, even 50 results may be insufficient and very large data sets are usually required to detect any interactions between factors. Therefore, the determination of overall worst-case scenarios, taking all of the main potential factors into account, may not be practically possible. Where worst-case conditions are predictable, e.g. tidal state, sampling dates and/or times should be allocated on as random a basis as possible (taking into account laboratory constraints, etc) within the periods where conditions apply. The annual sanitary survey review should include an assessment as to whether sampling has been undertaken under the worst-case conditions. If not, the sampling plan should be revised.

Explanation: Environmental factors, including season, tidal state (spring/neap), rainfall and wind have been shown to affect the degree of contamination of bivalve molluscs, as indicated by *E. coli*. The effects will vary from area to area and even point to point within an area. Bias towards one state of a factor may markedly affect the level of *E. coli* detected in samples. This will, in turn, affect any classification based on this data. Samples should therefore be taken on as random a basis as possible in order to even out these effects. As the intent of the legislation is public health protection, if this is not possible, it should be ensured that either the bias towards a state of a factor leads to the detection of the highest levels of contamination or alternative sampling points are selected which enable one or other approach to be satisfied. It should be noted that access to the bivalve molluscs, and associated sampler safety, may be affected by factors such as the state of the tide and this may affect the practical timing of sampling. Where this is the case, the sanitary survey should include an assessment as to whether there is any bias towards lower results as a consequence of the timing of sampling and the interpretation of data, and subsequent classifications, should take account of this in order to provide the equivalent level of public health protection as would have been given if such bias had not occurred.

3.14 Timing of sampling of relay areas

Recommendation: Samples for the classification monitoring programme should not be taken from a relay area until at least two weeks have elapsed since the depositing of the bivalve molluscs in the area. Where a relay area is divided into sub-areas for the purposes of batch operation, samples may be taken from a sub-area where this minimum time period has been satisfied.

Explanation: *E. coli* levels in bivalve molluscs deposited in a relay area may take several days to equilibrate to those characteristic of that area, depending on the species, the stress produced by the harvesting and deposition process, the seawater

temperature and the initial *E. coli* concentration. It is therefore necessary to delay sampling until the levels are likely to be characteristic of the area and a result of the original contamination. The minimum recommended period for such monitoring period does not relate to the relay period necessary to ensure *in situ* depuration of pathogens as many of these, particularly viruses, depurate at a much slower rate than do indicator bacteria such as *E. coli*. Any monitoring of relayed product for pathogens for the purposes of determining appropriate depuration periods, or whether the product is safe for consumption, is not covered by this Guide.

4. Sampling and sample transport

4.1 Introduction

Bivalve molluscs for the official microbiological monitoring of harvesting areas should be taken directly from those areas rather than being sampled from harvesters, or at purification or dispatch centres, in order to ensure that they have been taken from the designated sampling point(s) (as dictated by the sampling plan) and under the appropriate controlled conditions. Depending on the type of bivalve mollusc fishery, sampling may necessitate the use of a boat. Many of the harvesting areas are situated remote from both samplers' offices and from laboratories. The sampling time may be dictated by the accessibility of the area, often dependent on tidal cycles (except in the Mediterranean Sea). This may result in inconvenient times for sampling, sample transport and/or laboratory testing. All of these factors mean that sampling and sample transport need to be carefully planned and sufficient resources made available to ensure that the data obtained from the sampling programme is relevant.

Samples are obviously taken under field conditions, and the results of the sample analyses depend largely on sampling methods, spatial and temporal distribution, and analytical method. The sampling method and treatment of the sample during and after sampling are therefore important. This includes the packaging material which is used, transportation method, and duration and temperature control of the sample(s) between sampling and testing. This section will therefore focus on the main factors to be defined with regard to sampling and sample transport in connection with microbiological monitoring of harvesting areas. The section does not focus on identification of sampling point or frequency as this is covered in a previous section.

4.2. Sampling and sample transport protocols

Recommendation: Sampling officers should be provided with a protocol containing details as to how samples should be taken, cleaned of sediment, packed and transported. Where samples are taken with the help of the industry, e.g. if an official boat is not available, it is preferable for this to be done under the supervision of a sampling officer. If this is not possible, sampling protocols and relevant training should be provided and audits undertaken to ensure compliance with the protocol.

The following should form part of a sampling protocol:

a) The location and type of sample
b) The means of sampling
c) Number and minimum weight of individual animals forming the sample (by species)
d) Cleansing of the exterior shells of samples
e) Sampling record (perhaps on sample submission form)
f) Sample containers and outer packaging to be used
g) Temperature control during transportation
h) Acceptable time lag between sampling and analysis

It is advantageous, where possible, to sample using the means normally used for commercial harvesting as additional contamination may be introduced during some dredging procedures.

Explanation: Sampling and sample transport protocols are an important basis for ensuring the standardisation of these procedures and therefore that the results obtained from the samples are representative of the bivalve molluscs in the harvesting area. In order to ensure that the protocols are applied, they should be available to all involved in the management of the monitoring programme and the taking and transport of samples.

4.3 Sampling method

Recommendation: Wherever possible, species should be sampled by the method normally used for commercial harvesting. Where this is not possible, or where an indicator species is being used, samples may be taken by other means (e.g. hand-picked) or bagged bivalve molluscs may be kept at the sampling point for the purpose of sampling. With the latter, the effect of location in the water column should be considered (see Section 3.7). Where samples are taken other than by the method normally used for commercial harvesting, occasional samples should be taken from the commercial harvest (prior to any grading, washing or processing) in order to ensure that the results of the monitoring programme using a different means of sampling are valid.

Explanation: Commercial harvesting practices may disturb sediment, etc, which may be taken up by bivalve molluscs which are open and filtering. Any ingested sediment may contribute to the degree of contamination and the use of samples taken by other means may not be fully representative. Recommendations relating to the sampling approach for different types of bivalve mollusc fisheries are given in Annex 2.

4.4 Size of individual animals

Recommendation: Samples should only consist of animals that are within the normal commercial size range.

Explanation: Immature/juvenile bivalve molluscs may give *E. coli* results that are unrepresentative of mature stock that will be harvested for commercial sale/human consumption.

4.5 Number of animals per sample

Recommendation: The minimum number of individual animals per sample should be specified by the competent authority or other agency responsible for the monitoring programme management for each species. An allowance should be made for a proportion of animals being received by the laboratory in a moribund state. After such an allowance, at least 10 individual animals per sample should be available for testing at the laboratory, with the minimum amount of FIL from those animals being at least 50g, except in the case of *Donax* spp. where the minimum amount should be 25g.

Explanation: Variation in *E. coli* content of individual bivalve molluscs of the same species samples at the same site at the same time can be large. Increasing the number of animals tested per sample helps to average out this variation. Regulation (EC) No 2073/2005 specifies the use of a pool of a minimum of 10 individual animals. There is also a need to ensure that sufficient FIL is available for the test procedure.

Individuals of the *Donax* genus are very small and it is usually not practical to try to obtain 50g FIL from a single sample.

4.6 Avoidance of contamination

Recommendation: Equipment used for sampling should be kept for that purpose and be clean. Suspension of sediment should be avoided – where possible, samples should be taken upstream of any potential disturbance (such as the sampler). After the bivalves have been removed from the water and have closed, any mud and sediment adhering to them should be removed by rinsing/scrubbing with clean seawater or fresh water of potable quality. If these are unavailable the seawater from the immediate area of sampling may be used instead. Do not totally re-immerse the shellfish in water as this may cause them to open. Allow to drain.

Explanation: Sampling of bivalve molluscs needs special care from the sampler, since the animals may continue filter feeding until they are taken out of the water. Potential contamination before, during and after sampling should be avoided at all times. This can be achieved by using the proper equipment and proper cleaning of the sample. Bivalve molluscs covered with dirt, sediment, algae and other organisms may become contaminated inside the sample bag.

4.7 Sample bags and containers

Recommendation: Each sample should be placed in a separate intact food grade plastic bag. A waterproof label should be affixed to each bagged sample and should contain the following information: sample reference number, sample date and time and any other relevant information (e.g. species). This bag may be placed inside a second bag or other container.

Explanation: Placing samples in appropriate bags protects them from contamination and prevents them from cross-contaminating other samples and the transport containers. The use of proper labelling procedures ensures traceability.

4.8 Sample transport criteria

Recommendation: Sample transport criteria should conform to the requirements given in ISO 6887-3. This states that: “On arrival at the laboratory the internal air temperature of the transit container should be recorded. For samples where more than 4 hours have elapsed between collection from the production area and receipt, the internal air temperature should be between 0 °C and 10°C. If the internal air temperature is greater than 10 °C, the sample temperature should be measured; this should not exceed 10 °C. For samples where less than 4 hours have elapsed between collection from the production area and receipt, internal air temperature should be less than the temperature recorded at the time of sampling. Test portions shall be stored at 3 °C ± 2 °C and should be processed within 24 hours of collection. If initiation of the microbiological analysis cannot be within 24 hours of sample collection, data should be generated to show that extended storage does not affect the microbiological content of the sample.”. It is also recommended that verification studies should also be undertaken to support use of transport and storage temperatures outside of the ranges given in ISO 6887-3. Competent authorities should undertake, or initiate, such

verification studies and should approve any sample transport and storage requirements based on the outcome of these.

Explanation: The growth and/or mortality of micro-organisms in foodstuffs are related to both temperature and time. Presently available data indicates that *E. coli* will not significantly increase in mussels (*M. edulis*) or Pacific oysters (*C. gigas*) at temperatures of 15°C or less within 48 hours (Cefas, 2008; Doré W., *pers. commun.*). Freezing and subsequent thawing will reduce the *E. coli* count by approximately 1 $\log_{10}$ (Lart & Hudson, 1993).

4.9 Sample submission form

Recommendation: The following should be recorded on the sample submission form:

- sampling point identification number and name
- map co-ordinates (grid reference and/or latitude/longitude)
- time and date of collection
- species sampled
- method of collection (hand-picked, dredged, etc)
- seawater temperature (or air temperature for intertidal species exposed at time of sampling) .

Any other information deemed relevant (e.g. unusual events, adverse weather conditions etc) should also be recorded. One copy of the submission form should be kept for the sampler's records and another should accompany the sample to the laboratory. If electronic systems are used for this purpose they should record the same items of information and allow information to be retrieved by both the sampler and the testing laboratory.

Explanation: It is important to use appropriate sample submission forms in order to prevent loss of data, and to ensure traceability. An example form is shown in Table 4.1.

4.10 Sampling instructions

Recommendation: A set of instructions should be provided to, and available for reference by, all persons taking samples for the monitoring programme. This may simply be the protocol referred to in Section 4.2 or a subset of this protocol relevant to the operations undertaken by the specific sampler.

Explanation: Sampling procedures can introduce additional variability into the results or may even invalidate the use of results obtained. Use of standard procedures will reduce this and these must be available to, and regularly referenced by, the staff involved in taking samples.

4.11 Training of samplers

Requirement: All samplers should receive formal training before being allowed to submit samples to the monitoring programme. Requirements for training are stipulated in Article 6 of the Official Feed and Food Control Regulation (Regulation

(EC) No 882/2004). A unique identifying number should be allocated to each trained sampler in order to assist recording and reporting procedures. Samplers should also be provided with relevant sampling and safety equipment.

Explanation: Proper training of samplers is necessary to ensure compliance with the sampling protocol. Sampling in the marine environment is also hazardous and proper training is necessary from a health and safety perspective.

Table 4.1 Example of a sample submission form

Programme code/description	
Sampler's reference number	
Sampler's name	
Sample reference number	
Date	
Time	
Sampling point number	
Sampling point name	
Sampling point location (grid ref or lat/ long)	
Shellfish species	
Collection method (please circle)	Dredged Hand-picked Hand-raked Diver-gathered Other (please specify)
Tidal Phase (please circle)	Spring Neap
	High Ebb Low Flood
Water temperature (if shellfish covered)	
Air temperature (if shellfish exposed)	
Wind (direction and speed) [1]	
Rainfall in last 48 hours[1]	Yes / No
Observations[2]	
Lab arrival date	
Lab arrival time	
Accepted by lab (if No, please given reason)	Yes / No

Notes: [1]Optional at the discretion of the competent authority or other agency managing the monitoring programme

[2] e.g. Animals/Birds/overflows operating/vessels in area/tourists/etc.

4.12 Provision of samples by industry

Recommendation: Where officers of the competent authority, or other authorized official bodies, cannot obtain samples, members of the industry may provide them as long as the requirements of Sections 4.3 to 4.12 are met. Wherever possible, such sampling should be supervised by an authorized officer. Where this is not possible, occasional samples should be taken by an authorized officer or under supervision of such an officer. Procedures should be instituted to ensure that any possible

deviations from protocols are identified at the time of sample submission and not after the laboratory result is known.

Explanation: The microbiological monitoring programme forms part of the official controls for harvesting areas and it is essential that samples are taken from the designated point(s), according to the requirements of the sampling plan and handled and transported according to the sampling and sample transport protocol. Failure to do so may significantly affect the results and therefore the classification.

4.13 Provision of sample results by the industry

Recommendation: Where, as allowed in Regulation (EC) No. 854/2004, it is decided to take into account results obtained by industry outwith the official sampling and analysis system, the following considerations should be applied. The location(s) and timing of samples should be such as to adequately represent the level of contamination in the area and this should be assessed with respect to the outcome of the sanitary survey. Sampling and sample transport procedures should conform to protocols issued by the competent authority, or other agency managing the monitoring programme, and the recommendations of Sections 4.2 to 4.12 and 4.14 to 4.15 inclusive. Laboratory analyses should conform to the recommendations given in Section 5. A specific agreement should be established between the competent authority, or other agency managing the monitoring programme, and the testing laboratory in order to ensure that the complete set of results are made available. An example agreement is given at Annex 4.

Explanation: It is necessary to ensure that all data taken into account for the purposes of determining the classification status of an area is representative and of equivalent quality. It is therefore necessary to ensure that results submitted for consideration by the industry are based on samples taken at points and on occasions that reflect the contaminating sources, and are sampled, transported and analysed according to standard protocols. It is also important to ensure that a complete data set is taken into account in order to avoid biasing the subsequent data analysis one way or the other.

4.14 Audit of sampling and transport procedures

Recommendation: Sample recording procedures should include verification procedures to ensure that appropriate aspects (e.g. time-lag, temperature of sample) of the protocols have been met (see Section 4.2 to 4.9). Physical audits of the sampling and sample transport procedures should normally be undertaken at least once a year for each sampler (officer or industry) in order to ensure that the relevant protocols are being complied with. However, a risk assessment may be used, taking into account whether the sampler is from an official body or the industry, any previous problems shown at audit and any problems found with samples received at the laboratory. On the basis of an assessment of good control and compliance, the period between audits may be extended up to three years. Deviations from the protocols detected during the audit, or by other means such as condition of the sample on receipt at the laboratory should be rectified – this may require retraining of the individual sampler.

Explanation: *E. coli* levels measured in individual samples may be markedly affected by factors during the sampling and sample transport procedures. This will then affect the classification. It is therefore essential that there is an ongoing assessment of compliance with the protocols. Some aspects can be checked at the data entry stage for the samples. Further verification of these, together with other aspects, require physical audit of the sampling and sample transport procedures.

4.15 Receipt of samples by the laboratory

Recommendations:

a. Sample viability

Only viable animals should be homogenised for the *E. coli* test. The laboratory should therefore only test samples if the number of animals that are viable meets the minimum stipulated for the species (and at least 10 for any species) and the minimum weight of flesh and intravalvular fluid obtained from the viable animals is at least 50g (25g for *Donax* spp.).

b. Sample container

A sample must be received in an intact food grade plastic bag. The container/bag should be labeled with the sampler's reference number and any other relevant information (e.g. species). Samples should not be examined if they are received unlabelled or without a sample submission form.

c. Temperature on receipt

The laboratory should at least record the temperature of the samples on receipt to show that they are within the specified temperature range. It is preferable to include a continuous temperature recording device within the mass of the bivalve samples and to download and assess the temperature record prior to reporting the laboratory result.

d. Condition of sample

A sample is also considered unsatisfactory on receipt when:

- The sample bag is received leaking such as to lead to potential contamination of that or other samples
- The shellfish are immersed in water or mud/sand

If samples are received in a state in which they are considered unsatisfactory, a note should be recorded to this effect and the sending authority should be informed that this may be a factor affecting the quality of the result.

Explanation: These acceptance criteria provide simple checks on recommendations given earlier in Section 4 and thus enable compliance with those recommendations to be determined on an ongoing basis.

5. Microbiological testing

5.1. Introduction

Regulation (EC) No 854/2004 specifies the use of a five-tube, three-dilution Most Probable Number (MPN) method for the classification of class B and C areas. Regulation (EC) No 2074/2005 on implementing measures a further specifies the use of ISO TS 16649-3 as the reference method for class B and C areas while cross-reference from Regulation (EC) No 854/2004 to the Commission Regulation on microbiological criteria for foodstuffs also specifies this as the reference method for class A areas. ISO TS 16649-3 is based on the method of Donovan *et al*. (1998) and is a two-stage, five tube by three dilution MPN method (ISO 2005a). The first stage of the method is a resuscitation requiring inoculation of minerals modified glutamate broth (MMGB) with a series of diluted bivalve mollusc homogenates and incubation at 37±1°C for 24±2 hours. *E. coli* is subsequently confirmed by subculturing tubes showing acid production onto tryptone bile glucuronide agar (TBGA) and detecting β-glucuronidase activity by the presence of blue or blue-green colonies. ISO TS 16649-3 cross-refers to ISO 7218 for determination of the most probable number from the combination of positive and negative tubes (ISO 2013). Only tube combinations corresponding to categories 1 and 2 should be used to determine MPN results for samples taken official control purposes.

Methods for the preparation of samples can be found in EN ISO 6887-3:2003 (ISO 2003a).

It should be noted that, while year references are given at the time of preparation of this guide, ISO standards and technical specifications are subject to change and the most up-to-date version should be used. A standard operating procedure based on the current standards has been prepared by the EURL and is available at: https://eurlcefas.org/public-documents/methods.aspx. .

5.2 Dilution ranges

Recommendation: The dilution range prepared for each sample should be based on previous experience of the likely extent of contamination in an area. In areas where the extent of contamination fluctuates markedly, or the expected extent of contamination has not yet been fully determined, this may mean that four dilutions may need to be set up for an MPN test. The laboratory, or agency managing the monitoring programme, should therefore continually review the historic data for each point from which it receives samples in order to determine the correct range of dilutions to use in the test.

Explanation: A greater than (>) value does not give adequate information on the concentration of *E. coli* in the sample in question – the actual concentration may be markedly higher than the value quoted (e.g. a result reported as >18000 *E. coli* per 100 g could really be 63000 *E. coli* per 100 g, or even higher). Therefore, full assessment of the extent of contamination of a harvesting area cannot be undertaken if such values are present in the data set and it is necessary to prepare sufficient dilutions to enable an endpoint to be determined. Once sufficient historical data is available for a sampling point, the dilution series for ongoing use can normally be

determined. However, this may be affected by intermittent contamination events or step changes in the general level of contamination and this necessitates an ongoing review of the dilution series to be used.

5.3 Validation of alternative methods

Recommendation: The requirements of EN ISO 16140 (ISO 2003b) should be followed with the following clarification:

a. For international applications, or national applications where the method will be used by 8 or more laboratories: the full requirements of the interlaboratory trial section (6.3) in ISO 16140 should be followed.
b. For national, regional or local applications where the method is to be used by two or more, but less than 8, laboratories, the interlaboratory trial section should incorporate all such laboratories.
c. For regional or local applications where the method is to be used by a single laboratory, the interlaboratory trial requirements should be replaced by demonstration of fitness for purpose (EN ISO/IEC 17025).

Alternative *E. coli* methods for which the validation has been accepted as satisfactory by the EURL are:

i. Impedance method: EURL generic protocol - Enumeration of Escherichia coli in live bivalve molluscan shellfish by the direct impedance technique using Bactrac 4300 series analyser. Current issue. http://www.crlcefas.org/InformationCentre/docs/E_coli_enumeration_BacTrac_impedance_technique_v1_08_06_11_issue_01.pdf

ii. Colony count method: EURL generic protocol - Enumeration of Escherichia coli in bivalve molluscan shellfish by the colony count technique (based on ISO 16649-2). Current issue. http://www.crlcefas.org/InformationCentre/docs/Issue_2_EURL_SOP_E_coli_TBX_final.pdf

Protocols for these two methods can be found at: https://eurlcefas.org/public-documents/methods.aspx.

Where an alternative method has been validated according to a), subsequent application in other laboratories will only require demonstration of fitness for purpose in each laboratory.

Explanation: Alternative methods must be properly validated against the reference method in order to ensure that they will yield equivalent results. In general, the requirements of EN ISO 16140 for the validation of quantitative methods will ensure that this is met. However, the reference laboratory network has expressed concern that the requirements for interlaboratory studies in EN ISO 16140 are excessive when the alternative method is intended for use in a relatively small number of laboratories and this has resulted in the approach recommended above. It is intended that the requirements for different levels of validation will be determined during a revision of the standard.

5.4 Accreditation

Recommendation: All laboratories undertaking testing of bivalve molluscs under a competent authority monitoring programme (including those contributing results of samples taken by, or on behalf of the industry) must be accredited to EN ISO/IEC 17025 for the specific method used for *E. coli* in bivalve molluscs (ISO 2005b).

Explanation: The competent authority control programme is part of the official control system for harvesting areas. Laboratories undertaking official control testing must be accredited (Regulation (EC) No 882/2004). It is essential that all results included in the determination of classifications and other official monitoring of harvesting areas are based on data produced by laboratories working to such standards. This is one component necessary to ensure the comparability of results (in conjunction with others given below).

5.5 Internal Quality Control

Recommendation: Internal quality control procedures are specified in ISO TS 16649-3 and EN ISO/IEC 17025. Laboratories using alternative methods should include relevant positive and negative controls for each batch of tests in all stages of the procedure. Consideration should be given to the processing of quantitative positive internal controls on at least a weekly basis. For impedance systems, it is also essential that each impedance curve is checked for conformity and also against the characteristics of a known *E. coli* impedance signal.

Explanation: The use of appropriate internal quality control procedures is essential to ensure that the results from each batch of tests are valid. These controls are often positive/negative in nature. The use of quantitative internal positive controls on a regular basis provides an additional measure of the performance of enumeration methods. ISO 19036 gives guidance on the estimation of measurement uncertainty for quantitative determinations in food microbiology (ISO 2005c).

5.6 Comparative Testing

Recommendation: All laboratories undertaking testing of bivalve molluscs under a competent authority monitoring programme should take part in a relevant external quality assurance scheme and must participate in proficiency testing/ring trials for *E. coli* in bivalve molluscs organised by their National Reference Laboratory (NRL). Laboratories in third countries which do not have a designated NRL should request to participate in proficiency testing/ring trials organised by the European Union Reference Laboratory (EURL)(this would be subject to a charge by the EURL). Comparative testing should be undertaken at a minimum frequency of twice a year.

Explanation: Proficiency testing provides an independent assessment of the performance of a laboratory and allows this performance to be compared with that of others. The frequency has to be sufficient to allow the organisers to properly detect poor performance within a reasonable timescale. Proficiency testing supplements, and does not replace, the need for the requirements identified in Sections 5.1 to 5.5.

5.7 Supervision by the NRL

Requirement: The NRL must oversee the activities of all laboratories contributing data to the official microbiological monitoring programme. The NRL must ensure that the laboratories use the European reference method, or an alternative properly validated against this according to Section 5.2, that they are specifically accredited for this method (see Section 5.3), and participate in one or more appropriate proficiency testing programmes (see Section 5.5). The NRL must undertake periodic reviews of the performance of these laboratories in the proficiency testing programmes.

Explanation: The supervision of national laboratories by National Reference Laboratories is stipulated in Regulation (EC) No 882/2004. In order to ensure the quality of results produced by these laboratories, the NRL needs to verify that the requirements of Sections 5.1 to 5.6 of this guidance are met.

6. Data handling and storage

6.1 Introduction

Proper management of the microbiological monitoring programme, and subsequent analysis of the data, requires that the relevant information and results are stored in a secure, well-organised and easily accessible form. In general, the most effective and versatile way to achieve this is in the form of a relational database. Given that much of the information from the programme will have a geographical element, programme management and data analysis will be assisted if the database is linked to a GIS or the data is managed within the GIS itself. The recommendations are given below as if they are to be contained in a single data management system. While this is preferable, to aid ease of data retrieval, this does not preclude the capture of different parts of the data set in separate, or linked, systems.

6.2 Databases

Recommendations:

Storage Data from the monitoring programme should be stored in a secure database which has tables containing the following:

i) Information on the sampling plans (see Section 3.2)
ii) Information relating to the samples
iii) Results of the testing of samples

The following may also be considered for inclusion in the database:

i) Results of the sanitary survey
ii) Information on pollution events
iii) Results of investigations into pollution events and anomalous *E. coli* results

Security features In order to maintain the integrity of the data held within the system, access should be password protected and users are individually assigned read only or write permissions according to organisational need.

Data verification Mandatory data fields (e.g. sampling point identifier, species, date and time of sampling, temperature at time of receipt, date and time of start of test, *E. coli* result) should be checked after entry into the system. Automatic checking of some fields may also be undertaken (e.g. sampling point/species combination, delay between sampling and start of test, temperature within acceptable limits, *E. coli* result against class of area).

Retrieval of data Sampling plans should be accessible by both harvesting area and sampling point. *E. coli* results should be at least retrievable by sampling point and date range.

Data audit A traceability system should be introduced so that any changes to data are recorded together with an identifier of the person making the change and the reason therefore.

Integration with the mapping functions Where a GIS is used instead of hard copy maps, the general content of sampling plans should be available via the mapping functionality. This will necessitate the display of sampling points within an area with links to the sampling plan information. If sanitary survey information is stored within the system, then the sampling plan may form part of this more detailed information accessible via the mapping. Individual or summarised *E. coli* results may also be linked to sampling points and displayed via the mapping interface in numerical or graphical form.

Web-based data publication The Internet may be used to disseminate information from the monitoring programme in either a publicly accessible or password-protected form. Relevant parts of the data may be uploaded and accessed either by reference to a map of a relevant area or via a data selection tool. Information that may be relevant to distribute in this way are the sampling point locations, sampling plans and microbiological results.

Explanation: The microbiological monitoring programmes for Member States or Regions with more than a few fisheries will rapidly accumulate large amounts of data. It is important that this data is properly validated and is readily accessible and analysable. The use of a dedicated database, preferably linked to a Geographic Information System to enable proper display of geographical data, will enable these requirements to be achieved.

7. Interpretation of monitoring programme data

7.1. Introduction

As noted in the introduction, classification yields an assessment of risk of contamination based on the presence of faecal indicator bacteria and determines the subsequent treatment to which harvested bivalve molluscs must be subjected. Classification is based on historical time series data and provides a prediction of that risk of contamination for a period into the future. In this sense, there is no special interest in historical compliance in itself, only its use in predicting the risk.

The interpretation of the data from the monitoring programmes (by application of the sampling plans) established for the classification and surveillance of the production areas must consider, alongside the decision criteria given in the Regulations (see Table 1.1), other factors such as influence of environmental conditions, analytical variability, sampling point characteristics, sources of contamination and their characteristics, etc.

There is also the need to consider that there are a large number of external factors, generally environmental (rain, state of the tide, wind regime, bathymetry, estuary circulation, etc.), that increase the variability of the environmental monitoring data. The effect of these external factors can be reduced by using data sets containing large numbers of results obtained over time. It is therefore necessary, as far as possible, to avoid fluctuations in the classification of the production areas that are consequence of the effect of these external factors that can be pronounced when data sets are small or cover short time periods.

7.2 Delineation of classified zones

Recommendation: A classified zone should be defined by precise geographical limits (to an accuracy of +/- 10 m) enclosing an area of sea, estuary or other relevant body of water, and, where relevant, identifying where the zone meets the coastline. It should ideally be homogeneous with respect to the following: access, production activity, demarcation, hydrographic features and characteristics of the circulation of microbiological pollutants. The zone may cover all, or part, of a production area. A relay area should be completely included in a single zone. Where all of these characteristics are not met, the deciding factor as to whether to have a single or multiple zones should be whether the area constitutes a single entity from the viewpoint of enforceability by the competent authority (See also Section 3.4). There should be at least one sampling point in each zone.

Explanation: It is necessary to clearly define the limits of a classified zone in order for the sampling plan to be regarded as representative, to assist in the analysis of data, and to allow subsequent enforcement by the competent authority. It is necessary to take into account the outcome of the sanitary survey and the microbiological monitoring for each area in deciding the extent and limits of the zones.

7.3 Interpretation of monitoring programme data

7.3.1 Initial classification

Recommendation: The results of 12 samples taken over at least a 6-month period (6 samples over 3 months for an area identified as remote) should be assessed for compliance with the criteria given in the legislation (see Table 1.1). See Section 3.8 for the sampling recommendations relating to initial classifications.

Explanation: While the results used for an initial classification will not reflect the full range of annual, seasonal or other variability that may be seen, there is a need to take sufficient samples over a period of time in order to take some account of the variability that will be seen in the results.

7.3.2 Primary established classification

Recommendation: Data obtained from the sampling should be reviewed on an ongoing basis in order to determine whether the initial classification should continue to apply. After one year, an established classification should be determined according to the criteria given in the legislation (see Table 1.1). See Section 3.9 for the sampling recommendations relating to primary established classifications.

Explanation: Data obtained between the initial classification and the primary established classification will generally be limited in terms of sample numbers and will not have been taken over a sufficient period of time to show the full extent of annual or other temporal variability.

7.3.3 Frequency of review of monitoring data – established classifications

Recommendation: Results from each sampling point should be reviewed on an annual basis, taking into account the last 3 years' data, or all data if less than 3 years' worth is available. An established classification established on this basis of this should normally last at least one year. A review should not be undertaken if there are less than 24 results available for 3 years or the appropriate proportion of this number if the period is less than 3 years. In such a case the classification of the area should be suspended until sufficient additional samples have been taken at the intervals prescribed in Section 3.11 or 3.12, as appropriate. For remote areas, a review should not be undertaken if there are less than 12 results over a 3-year period, or part thereof, and the classification of the area should be suspended until sufficient additional samples have been taken at the intervals prescribed in Section 3.11. The results should be spread over the period in question. Where no results are available for sampling occasions identified within the sampling plan, the reasons for the absence of results should be explicitly documented.

Explanation: The effect of variation in the concentration of faecal indicators in the polluting sources, together with the variability in the way that environmental factors affect the way that the sources impact on the microbiological quality of the bivalve fisheries, means that a proper assessment of the status of areas can only be made on the basis of a relatively large number of samples spread over a length of time and environmental conditions. Three years is considered to be the minimum period over

which much of the range of variability may be seen – this will vary from area to area but cannot be judged prior to the acquisition of monitoring results.

7.3.4 Potentially significant changes in known sources of faecal contamination affecting an area with an established classification

Recommendation: A review of the sanitary survey should be undertaken in order to determine whether the previously determined sampling points are still valid. Data obtained since the changes in contamination sources should be assessed as for an Initial Classification (see Section 7.3.1).

Explanation: The potential significance of any changes in the number, location and nature of sources of faecal contamination will need to be assessed with respect to the information on known sources and hydrography presented in the sanitary survey, together with the distance from the shellfishery(ies). A significant change in one or more known sources of faecal contamination may affect both the general level and spatial distribution of *E. coli* at the shellfishery. Previously established sampling point(s) may no longer properly represent the contamination status of the shellfishery. Monitoring data obtained prior to the known change(s) will not represent the existing situation and thus any classification based on that data may either not provide the appropriate level of public health protection (if contamination has significantly increased) or may cause the area to be classified at worse level (e.g. C instead of B) than that which would reflect the current microbiological status (if contamination has significantly decreased).

7.3.5 Interpretation of data in a zone with a single sampling point

Recommendation: The data set recommended in 7.3.3 should be assessed for compliance with the requirements in the legislation (as given in Table 1.1).

Explanation: The criteria for each class of harvesting area (A, B or C) are given in the legislation. No allowance for analytical uncertainty is currently given for those criteria. A flow diagram for the analysis of data is given in Figure 7.1.

7.3.6 Interpretation of data in a classification zone with several sampling points

Recommendation: Where multiple sampling points are used to represent a single classification zone, usually because of the presence of multiple contaminating sources, the results from each point should be assessed on the basis of the criteria given in Table 1.1. If a difference is seen between the points, the classification for a species in a zone should be based on the worst classification obtained from all of the sampling points (i.e. the most contaminated) for that species or the indicator species by which it is represented.

Explanation: While it is ideal for a classification zone to be homogenous from the viewpoint of the extent of contamination, in many zones it is likely that there will be multiple sources of contamination and differing effects of currents and environmental factors across the zone. In addition, it may be the case that the zone represents the smallest unit from which the competent authority or other control body is satisfied that the origin of the bivalve molluscs can be adequately monitored and assured. In such cases, in order to ensure public health protection, it is necessary to base the

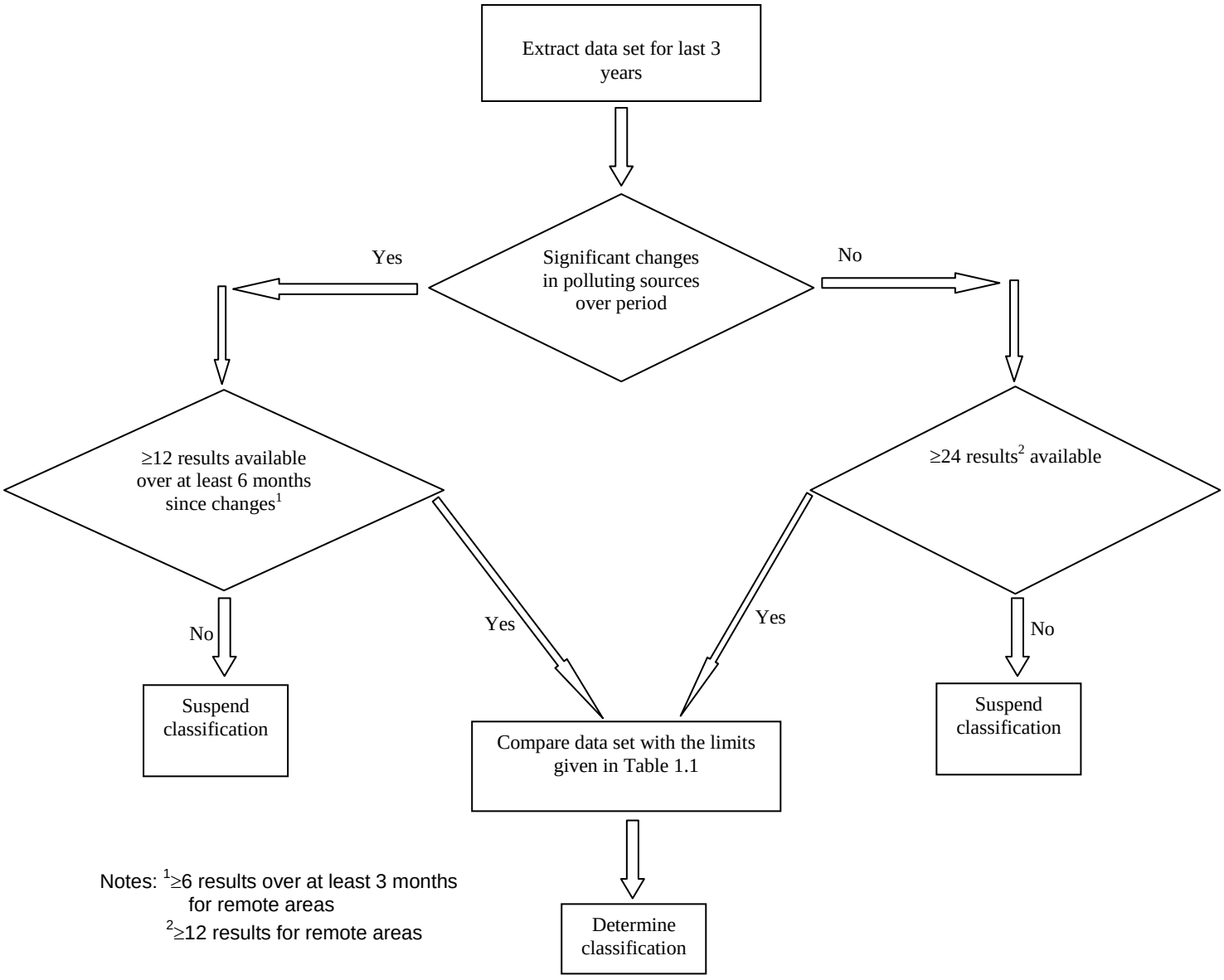
classification (and thus the required treatment post-harvesting) on the sampling point showing the worst classification for the species or its indicator species.

7.3.7 Effect of environmental factors

Recommendation: In zones where the trend of results has been shown to be markedly affected by either individual rainfall events or the total annual rainfall, and the most recent two years' have had significantly lower annual rainfall than average, the number of years to be included in the analysis should be extended by two.

Explanation: Differences in rainfall between years can markedly affect the results obtained from the microbiological monitoring programme in some areas. Given that the historical results are used to predict future potential risk of contamination, the extent of this could be underestimated if there are more results from years with significantly lower rainfall in the data set used for classification than results from normal or wet years.

Figure 7.1 Data interpretation for classification of harvesting areas



Notes: [1]≥6 results over at least 3 months for remote areas

[2]≥12 results for remote areas

7.3.7 Anomalous results

Recommendation: Results due to the following events may be identified as anomalous and excluded from the dataset used for determining classification status:

1) Failure to comply with the sampling protocols (e.g. temperature or time requirements not complied with)
2) Failure of the sewerage or sewage treatment systems that have been rectified and where the authority responsible for controlling pollution identifies that such a failure is not expected to recur
3) A rainfall event with a return period of 5 years or greater (i.e. rainfall of that intensity/duration which is only likely to occur once every five years or longer – this varies from location to location).

Where the authority responsible for the monitoring programme deems that:

a) a failure with regard to item 1 may have significantly affected the microbiological result;
b) an occurrence of items 2 or 3 have, or may have, significantly impacted on the microbiological status of the harvesting area.

With respect to item 1, an additional sample should be included in the sampling plan for the year on a random basis. For this criterion, all results (low as well as high) should be excluded from the dataset.

With respect to the occurrence of items 2 or 3, consideration should be given to the taking of further investigative samples and to the imposition of short-term control measures on the harvesting area.

Explanation: Criteria given in the sampling protocol are intended to ensure the validity of, and reduce variability in, the microbiological results. Significant deviation from the criteria may mean that the result obtained may be significantly higher or lower than the actual concentration in the bivalve molluscs at the sampling point at the time of sampling.

Failure of the sewerage or sewage treatment systems, or truly exceptional rainfall may give abnormally elevated *E. coli* concentrations in impacted harvesting areas. If these are unlikely to recur then including the results in the classification assessment (and therefore assessment of risk) of the area will not reflect the lower extent of contamination expected to occur over the forthcoming period of time. However, there may be chance associations of potential contamination events and high results with respect to time and it is necessary for a formal assessment to show that the high result is causally connected to the event. Investigative sampling immediately after a contamination event or a high result may aid identification of such a link and weekly sampling after the event (outside of the normal sampling plan) may help to identify when the microbiological status has returned to normal (but see Section 7.3.9).

7.3.8 Seasonal classifications

Recommendation: At least 2 years worth of data showing a clear seasonal trend is necessary to establish a seasonal classification. The season classified as the least

contaminated must be preceded by 2 months *in situ* relay period after a class C period or 1 month after a class B period, i.e. the historical results during this *in situ* relay period must also conform to the improved classification category. The required data set identified in Section 7.3.3 (in terms of numbers of samples and years of monitoring) should be available from sampling undertaken during the season and *in situ* relay period. A reduced frequency of monitoring cannot be applied to seasonal classifications. For areas that comply with class C during the more contaminated part of the year, sampling should be undertaken on a monthly basis during that period to preclude the occurrence of results >46000 *E. coli* per 100g of F.I.L. Where historical data, or the outcome of the sanitary survey, indicates that this is a risk in other areas, the same approach should be taken.

Explanation: Apparent differences in the extent of contamination can be seen in short term monitoring – this may be due to short term changes in the effect of environmental factors or even simply due to random variation in the data. It is therefore necessary to formally show (preferably using statistical analysis) that a difference in the extent of contamination exists between the differently classified parts of the year. It is also necessary to take sufficient samples during the active season (and *in situ* relay period) to enable the same assessment of the data as would occur if a single classification was extant year round. There is a need to ensure that contamination accumulated by the bivalves during the more contaminated months of the year is cleared before the better classification starts. The *in situ* period specified for a C/B or C/A area is that directly required by the Regulations for relay of class C bivalves. The lesser period of 1 month for a B/A area recognizes that the starting level of contamination in bivalves from class B areas should be lower than in those from class C areas. Continued monthly monitoring of areas during a class C period will rule out the possibility that a change in the extent of contamination has meant that the requirements for class C are no longer met – if this is the case then public health protection would dictate that harvesting from the area would need to be Prohibited. By the nature of the fluctuations in contamination seen in areas given seasonal classifications, applying a reduced monitoring frequency is not applicable as sufficient data must be obtained to support the classification of each respective season.

7.3.9 Alert monitoring procedures

Recommendation: If the following values are exceeded at a sampling point:

Class A: 230 *E. coli*/100 g of F.I.L.
Class B: 4600 *E. coli*/100 g of F.I.L.
Class C: 46000 *E. coli*/100 g of F.I.L.

or if a pollution event or extreme adverse weather conditions have occurred in an area, or if information is received regarding the association, or possible association, of the harvesting area with an outbreak of illness, then an alert procedure should be initiated. This should involve:

- investigative sampling instigated as soon as the result is known
- further sampling at weekly intervals
- pollution event investigations
- consideration of short-term controls to protect public health

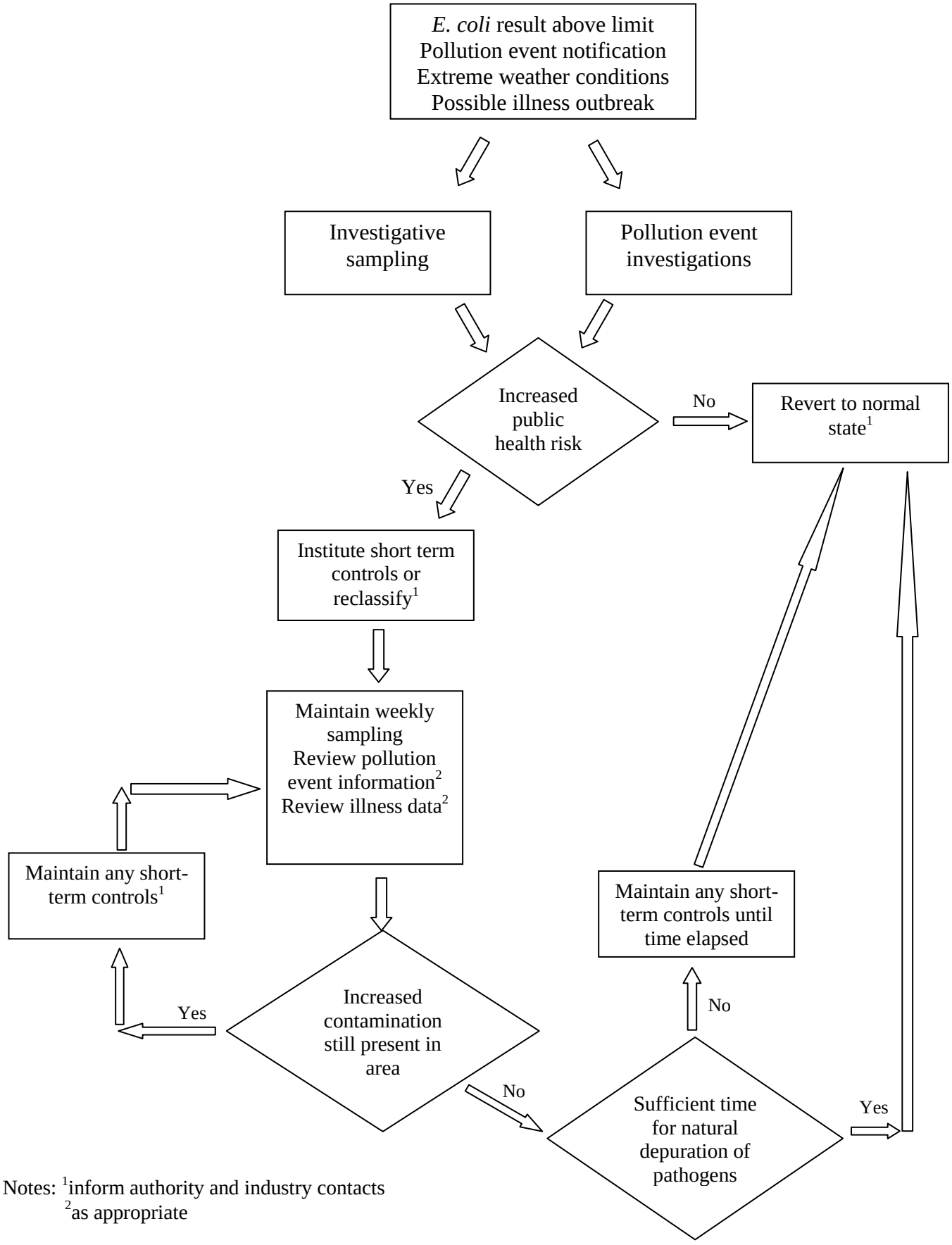
Relevant official and industry bodies at the national, regional and local level should be informed of the result, proposed action and outcome of the alert state. Where investigations indicate that the existing classification status is not consistent with the monitoring data, the classification should be revised accordingly. See Figure 7.2 for an example flow diagram for the alert procedure.

Where results from own-checks monitoring by the industry at dispatch or purification centres (or the results of audit samples taken by the competent authority) indicate that harvested batches have *E. coli* levels that exceed the above limits for the class of area, the alert procedures should also be invoked.

The results of any investigative and/or industry own-checks samples taken at dispatch/purification centres should not be taken into account for longer term determination of classification status. However, the results of all samples taken in accordance with the sampling plan for the area should be taken into account for the determination of classification status, including any taken during a closure period, unless they are identified as meeting the criteria for anomalous results given in Section 7.3.7.

Explanation: Single samples exceeding the limits specified in Regulation (EC) No 854/2004 indicate that the level of contamination in the particular class of area may be of direct concern to public health. Further results exceeding such limits could result in the classification being reviewed and downgraded. Investigative sampling will enable the extent of contamination to be ascertained and possible sources identified (and, where possible, rectified). Where a risk assessment shows that the public health concern is immediate, the taking of investigative samples should not delay consideration of short-term controls. The duration of any short-term controls, and interpretation of the results of investigative sampling, should take account of the differences in characteristics between the faecal indicator bacteria and the pathogens, especially viruses. In particular, the marked difference in time for depuration of the indicators and pathogens in the natural environment should be taken into account. For example, present evidence suggests that clearance of norovirus from Pacific oysters in the natural environment may take more than four weeks (EFSA, 2012).

Figure 7.2 Alert monitoring procedures - Example flow diagram



Notes: [1]inform authority and industry contacts
[2]as appropriate

8. Bibliography

Note: Specific editions of ISO standards are given below as they are the source of wording referenced in this document. It should be noted, however, that the current version of a standard should be used and this may be a later version than that referenced.

Anon. 2003. Report (SANCO/0064/2003 – rev. 3) to the standing committee on the food chain and animal health on the relationship between analytical results, the measurement uncertainty, recovery factors and the provisions in EU food and feed legislation with particular focus on the community legislation concerning contaminants in food (Council Regulation (EC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food), undesirable substances in feed (Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on the undesirable substances in animal feed).

Anon. 2003. Report (WD SANCO/58/2003) upon measurement uncertainties in microbiological analyses with a special reference to the uncertainties linked to the proposed microbiological criteria in the document SANCO/4198/2001.

Cefas 2008. The effect of time and temperature on the *Escherichia coli* content of live bivalve molluscs. Report by the UK National Reference Laboratory for monitoring bacteriological and viral contamination of bivalve molluscs.

Donovan T D, Gallacher S, Andrews N J, Greenwood M H, Graham J, Russell J E, D. Roberts D & Lee R 1998. Modification of the standard UK method for the enumeration of *Escherichia coli* in live bivalve molluscs. *Communicable Disease and Public Health* 1:188-196.

EFSA 2012. Scientific Opinion on Norovirus (NoV) in oysters: methods, limits and control options. EFSA Panel on Biological Hazards (BIOHAZ). European Food Safety Authority, Parma, Italy.

European Commission 2006. *Guidance document on official controls, under Regulation (EC) No 882/2004, concerning microbiological sampling and testing of foodstuffs.* Directorate General of Health & Consumer Protection, Brussels, 13 November 2006. 26pp.

European Commission 2014. Community Guide to the Principles of Good Practice for the Microbiological Classification and Monitoring of Bivalve Mollusc Production and Relaying Areas with regard to Regulation 854/2004. European Commission DG Health and Consumers. Issue 2.

European Communities 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Off. J. Eur. Communities* L 327, 22.12.2000: 1-72.

European Communities 2004. Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure

the verification of compliance with feed and food law, animal health and animal welfare rules. *Off. J. Eur. Communities* L 165, 30.4.04 : 1-141.

European Communities 2004. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for food of animal origin. *Off. J. Eur. Communities* L 226, 25.6.04 : 22-82.

European Communities 2004. Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. *Off. J. Eur. Communities* L 226, 25.6.04 : 83-127.

European Communities 2005. Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Communities* L338, 22.12.05 : 1-26.

European Communities 2005. Commission Regulation (EC) No 2074/2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004. *Off. J. Eur. Communities* L338, 22.12.2005 : 27-59.

European Communities 2006a. 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. *Off. J. Eur. Communities* L 064, 04.03.2006 : 37-51.

European Communities 2008. Commission Regulation (EC) No 1021/2008 amending Annexes I, II and III to Regulation (EC) No 854/2004 of the European Parliament and of the Council laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption and Regulation (EC) No 2076/2005 as regards live bivalve molluscs, certain fishery products and staff assisting with official controls in slaughterhouses. *Off. J. Eur. Communities* L227, 18.10.08 : 15-17.

Garreis M J 1994. Sanitary surveys of growing waters. In: Hackney C R and Pierson M D. *Environmental Indicators and Shellfish Safety.* pp 289-330. New York: Chapman and Hall.

ISO 2003a. EN ISO 6887-3. Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 3: Specific rules for the preparation of fish and fishery products. International Organization for Standardization, Geneva.

ISO 2003b. EN ISO 16140. Microbiology of food and animal feeding stuffs — Protocol for the validation of alternative methods. International Organization for Standardization, Geneva.

ISO 2005a. ISO TS 16649-3 – Microbiology of food and animal feeding stuffs – Enumeration of β-glucuronidase positive *Escherichi coli* – part 3 : Most Probable Number technique using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide acid. International Organization for Standardization, Geneva.

ISO 2005b ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories. International Organization for Standardization, Geneva.

ISO 2005c. ISO/PDTS 19036 – Microbiology of food and animal feeding stuffs –Guide on estimation of measurement uncertainty for quantitative determinations. International Organization for Standardization, Geneva.

ISO 2013. ISO 7218:2007/FDAM 1:2013, Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations - Amendment 1. International Organization for Standardization, Geneva.

ISO 2014. Microbiology of food chain -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -- Part 3: Specific rules for the preparation of fish and fishery products. International Organization for Standardization, Geneva.

Lart WJ and Hudson SA 1993. Factors affecting Escherichia coli levels in shellfish with reference to E.E.C. Directive 91/492. Report No. 419. Seafish Industry Authority, Hull.

Lees D 2000. Viruses and bivalve shellfish. International Journal of Food Microbiology 59:81-116.

NMKL Procedure N° 8 (1999) Measurement of uncertainty in microbiological examination of foods.

Rippey S R 1994. Infectious diseases associated with molluscan shellfish consumption. *Clin. Microbiol. Reviews* 7:419-425.

Annex 1. Example Shoreline Survey Form

	Shoreline Survey			
	General Information			
	Surveyor(s)	indicate as appropriate		
	Date of Survey	dd/mm/yy		
	Start Time	hh:mm		
	End Time	hh:mm		
	Location of survery area	name		
	Extent of survey area	from - to		
	Map	Map Number		
	Hydrographic Chart	Chart Number		
	Relative position of bivalve mollusc beds & access	Chart contour shore & access details		
	Location / proximity of shellfish water sampling point(s)			
	Location / proximity of bathing water sampling point(s)			
	Location / proximity of nearest bivalve mollusc sampling point(s)			
	Predicted Tides			
	Indicate source			
	HW	Time		
	HW	Ht (m)		
	LW	Time		
	LW	Ht (m)		
	Any unusual observed tidal conditions			

	WEATHER			
	Indicate source			
	Wind Direction, Strength	Beaufort Scale		
	Nearest Raingauge	Location Name & Grid Reference		
	Precipitation 48h preceeding survey	State source		
	Precipitation during survey	State		
	River flows	State relevant gauging stations with grid references and indicate if info to be obtained		
	KNOWN POINT SOURCE INPUTS			List with confirmatory grid references - indicate if from map (GR) or GPS fix (GPS)
KNOWN POINT SOURCE INPUTS	Streams & Springs	from Map or Hydrographic Chart		
	Known Discharges (e.g. from database)	List most relevant point sources		
	Known Discharges Observed at time of survey			
	Non-database Discharges Observed at time of survey	Sewage treatment works, Combined Sewer Overflows, Storm overflows, CSO's, SO,		
	Additional non-database Inputs Observed at time of survey	Culverts		
		Outfall pipes		
		Other – state		
	Toilet blocks, likely unsewered dwellings etc			
	Other comments on point sources			

	HARVESTING AREA INFORMATION			
		Harvesting Area		
		Nearest Bed(s)		
		Harvesting equipment		
		Evidence of harvesting activity		
		Access to Racks / beds on survey day		
		Access to Racks / usual state of tide / method		Variable/Spring Tide / Neap Tide/ High Water/ Low Water Hand picked / raked / dredged / diver /
	BIVALVE MOLLUSC MONITORING			
		Note names & changes in sampling personnel		
		Usual tidal state when sampled		Variable/Spring Tide / Neap Tide/ High Water/ Low Water
		Usual time of sampling		a.m. / p.m. / eve / Mo / Tu / Wed / Th / Fr / Sa / Su / Variable tides / OTHER:
		Usual Frequency of sampling		
		Note sample collection source		direct form Beds / dedicated sample Bags / Tressle bags / ropes / dedicated ropes
		Flesh Monitoring sample collection comments		
	BOATS			
		Boat Moorings		
		Boats, Work Boats		
		Boats, Fishing		
		Boats, Commercial		
		Other Vessels		

	SHORE OBSERVATIONS			
	For animals and birds give approximate numbers where possible	Animals on beach / shore		
		Estuarine animal presence - seals, birds		
		Strandline litter		
		Strandline sewage related debris		
		Water appearance / slicks, algal blooms		
		Beach signage - water quality information		
		Beach signage - e.g. dog bans, shellfish gathering / public health prohibitions		
	CATCHMENT OBSERVATIONS			
		Population estimate		
		Topography (e.g. steep-sided valley, flat land, etc)		
		Adjacent land use and catchment land use eg catchment grazing, forestry, arable		
		Wider Catchment land use eg catchment grazing, forestry, arable, other activities		
	PHOTOGRAPHS			

		Note time and position for each		
	SAMPLE COLLECTION			
		Date / Time / Sampler Initials		
		Media sampled		Water / Effluent / Shellfish / Sediment
		Sample location(s) name and reference points		
		Sample coordinate indicate if from map (NGR or lat/long) or GPS fix (GPS)		
		Depth sampled		
		Microbiological Analysis		
		On-site parameters:		e.g. Temp. DO, Sal, Turbidity, TDS
		Sample Site Comments tide,		river stream flow,
	OTHER COMMENTS			
		Industry details etc		

Annex 2. Sampling strategies for specific types of bivalve mollusc fisheries

Trestle culture

As plastic mesh bags are fixed on the trestles, sampling is often most convenient at low tide (sometimes at low spring tide) which may favour a worst case approach, depending on the siting of the trestles in relation to contaminating sources. Bivalve molluscs produced on trestle may also contain some sediment around them so the sample collected should be cleaned by rubbing sediment off by hand, if necessary, and properly rinsed with clean seawater or potable water.

Rope culture

Sampling of rope culture bivalve molluscs does not require any supplementary hygiene procedures. The rope is brought to surface for sampling and the depth, where appropriate, estimated from the surface point on the rope. The sampling plan may specify a specific depth. In this case, sufficient animals should be taken from as near to that depth as possible, usually within 0.5 m. Otherwise, the bivalves are collected from various depths of the rope according to the following procedure.

The amount of bivalve molluscs to be collected per meter depends on the length of the rope. A rope of 5 meters requires a standard sample of 20 animals (depending on mussel size), which means 4 animals per meter. This approach will average out any vertical spatial differences but may need to be modified if large variations in contamination are seen at different depths. In general, rope cultures do not contain large amounts of sediment, therefore the bivalve molluscs may be rinsed with clean seawater or potable water.

Pole culture (bouchots)

Like rope cultures the pole mussels should be sampled either at a specific depth, if defined in the sampling plan, or evenly over the length of the bouchot. Samples are best taken at low tide, since this is more practical, and the changes on picking up contaminations are (depended on the area), greater at low tide (usually greatest at low-water spring tides). Pole-grown bivalve molluscs usually lack large amounts of sediment; therefore no special attention should be taken for sediment removal. However, the bivalves need to be rinsed with clean seawater or potable water.

Sea-bed culture

Sampling of sea-bed grown bivalve molluscs requires special attention, since the fishery usually co-occurs with a heavy (contaminated) sediment load. The bivalve molluscs may be collected with use of a dredge. The bivalves are required to be brought to the surface, where they are properly pre-rinsed by submerging the dredge in the water in order to remove excess sediment. The remaining sediment needs to be removed with use of clean seawater, or preferably potable water. In order to take spatial variation into account, it is important to collect shellfish from different locations in the dredge.

Lot sampling

When a monitoring system is used where bivalve molluscs are collected from a fished batch the actual location of fishing needs to be recorded. It is also important to ensure that the delay between fishing and the start of the laboratory analysis does not exceed the period defined by the competent authority.

Tray culture

Bivalve molluscs produced on trays may contain sediment around them; therefore special attention should be taken to prevent contamination of the animals. When sampling at high tide, the tray should be lifted from the bottom (or installation). When surfaced the tray should be submerged several times to remove excessive sediments. If sampling occurs during low tide, the gross sediment should be removed by hand before the outsides of the bivalves are properly rinsed with clean seawater or potable water.

Wild shellfisheries

Wild bivalve molluscs may be collected using a dredge, raking or may be hand picked. Special attention should be taken to avoid contamination by sediment. Any sediment should be rinse of by submerging the dredge several times, or by rubbing the sediment off by hand (any which is applicable). The samples need to be rinsed with clean seawater or potable water.

Dredging

Bivalve molluscs growing on the bottom may be collected either by hand (digging) or with use of a hand operated dredge. Towing the dredge apparatus, followed by the collection of sample from the dredge, collects the shellfish. Since the dredge contains surface sediment, it is important that the shellfish are rinsed properly prior to packaging.

Raking

Cockles in the intertidal area may be collected at low tide by raking the sediment to a depth of about 3cm. Accumulated cockles should be picked out of the raked material on a regular basis to avoid possible sediment uptake and then rinsed and placed in the sample bag.

Hand picking

Hand picking of bivalve molluscs should be performed with consideration of post sampling contamination, therefore the animals should be collected with proper hygienic considerations.

Some species, such as scallops, razor clams, echinoderms and tunicates, may be hand-picked by diving. Care should be taken to avoid disturbing the sediment in the area as far as possible and to return the animals to the surface as soon after collection as is possible. Additional safety requirements will apply for this procedure.

Intertidal sampling

Samples from the intertidal area may be collected by dredging, hand picking or raking depending on the species and time of sampling.

Annex 3. Example agreement between the competent authority (or other agency responsible for the microbiological monitoring programme) and a laboratory analysing samples on behalf of a food business operator

Date: day, month, year
Authority reference number:
Laboratory reference number:

Agreement between [the Laboratory] and [the Competent Authority (or other agency)] relating to the provision of data for *E. coli*

[The Laboratory] herewith enters into an agreement relating to the performance of analyses for the presence of *E. coli* on the below given conditions.

§ 1

The Agreement covers samples which are taken by and forwarded by harvesters or other food business operators in relation to the analysis of bivalve molluscs for *Escherichia coli* where the results of the analysis are used in connection with the classification of the production areas established by the competent authority in accordance with food hygiene legislation.

§ 2

[The Laboratory] is accredited in accordance with the ISO 17025 to carry out the analysis for the presence of *E. coli* in bivalve molluscs in accordance with the methods given in the food hygiene legislation.

In addition [The Laboratory] must follow the instructions concerning the analytical methods given by [the Competent Authority (or other agency)] and by the [The ….. National Reference Laboratory for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs (hereafter called NRL)].

§ 3

[The Laboratory] must ensure that samples of bivalve molluscs are received, handled and analysed in accordance with requirements given in the food hygiene legislation and the recommendations given in the EU Good Practice Guide to the Microbiological Monitoring of Bivalve Mollusc Harvesting Areas.

§ 4

[The Laboratory] must report the results of all analyses undertaken for the purposes identified in §1 to the [the Competent Authority (or other agency)] by e-mail……. or alternatively by Fax …., as soon as the results are available. Each report must include information as to the accreditation status of the laboratory (meaning the seal of the accreditation body and the number of accreditation) and reference to the analytical method used.

The reporting of single or multiple results must be undertaken electronically by the use of a spreadsheet, which is designed by [the Competent Authority (or other agency)], and which is

given in annex A, unless [The Laboratory] has made another agreement with [the Competent Authority (or other agency)].

§ 5

[The Laboratory] must participate in a proficiency testing programme specifically intended for laboratories carrying out *E. coli* analysis on live bivalve molluscs under the food hygiene legislation. Such a program is offered by the English ”Health Protection Agency”, HPA, and is named ”HPA Shellfish EQA Scheme”. Further information and a registration form can be found on:

http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1200055669446?p=1200055669446,

or by contacting [The ….. National Reference Laboratory for Monitoring Bacteriological and Viral Contamination of Bivalve Molluscs (hereafter called NRL)]. In addition [The Laboratory] must participate in other proficiency testing programmes or interlaboratory comparisons organised by the NRL.

During participation in these proficiency testing programmes, [The Laboratory] must use the methods of analysis for *E. coli* in bivalve molluscs specified in § 3. [The Laboratory] must forward documentation to the NRL concerning the registration and participation in the proficiency testing programmes. After each distribution, [The Laboratory] must forward the results of its analyses plus the individual laboratory evaluation report to the NRL.

§ 6

The agreement can be annulled by both of the two parties with notice of 12 months. The agreement can further be annulled with immediate effect, if legislative, structural or similar conditions result in substantial changes in the existing conditions of the agreement.

§ 7

The [the Competent Authority (or other agency)] will not be responsible for any expenses connected with receiving, handling and analysing those samples mentioned in § 1, no. 1 and § 5 or the registration and reporting of analytical results of such samples.

Date:

for [the Competent Authority (or other agency)] for [The Laboratory]:

_____ _____

Annex A
Spreadsheet for use for the reporting of analytical results to [the Competent Authority (or other agency)]

Production area	Sampling							Sample receipt		Laboratory analysis			
Number and name of the production area	Form of harvesting (Wild fishery or aquaculture)	Identification number of the vessel or aquaculture establishment	Latitude or northing (Map reference) of sampling position	Longitude or easting (map reference) of sampling position	Species of bivalve molluscs	Date and time of sampling	Temperature at time of sampling (seawater for immersed bivalves, air temperature for exposed)	Date and time of receipt on laboratory	Temperature of sample on receipt at the laboratory	Date and time of start of analysis of analysis	Method of analysis	Number of analytical report	Analytical result *E. coli*/100g
196 Damhussøen	Mussel line	B-58	00° 00'.0	00° 00'.0	*Mytilus edulis*	26.09.05 14.15	15°C	27.09.05 09.15	6°C	29.09.05	ISO 16649-3	M369	<20
126	Dredged	Kaj Anker F-5896	00° 00'.0	00° 00'.0	*Crassostrea gigas*	24.12.05 16.30	12°C	25.12.05 09	8°C	27.12.05	ISO 16649-3	M370	<20

Annex 4. Additional requirements for production areas from which LBMs are harvested for export to the USA

A4.1 Introduction

The detailed background to these additional requirements is given in Annex 2 to the Community Guide (European Commission, 2014). The Annex to this Technical Guide contains recommendations as to how to achieve the requirements for buffer zones around wastewater discharges and marinas.

Buffer zones around point source inputs of human wastewater (such as sewer pipes or marinas), where harvesting is not permitted, are an explicit requirement of the US National Shellfish Sanitation Programme Manual of Operations (NSSP MO) (FDA, 2013). Their designation is a preventative public health measure principally aimed at protection against contamination of molluscs with human enteric viruses such as norovirus and hepatitis A virus. Their designation reflects the fact that routine faecal indicator monitoring cannot necessarily be relied upon to indicate the public health risk in such circumstances - particularly where the discharge is of treated effluent. It is well established that faecal indicator bacteria have different survival characteristics to enteric viruses both during sewage treatment processes and in the marine environment. Such buffer zones are not currently an explicit requirement of EU legislation but may be considered to be covered by the general provision in EU 854/2004 (Annex II, chapter II: C.1) that 'where the results of sampling show that the health standards for molluscs are exceeded, or that there may be otherwise a risk to human health, the competent authority must close the production area concerned, preventing the harvesting of live bivalve molluscs'.

It is important to note that aspects of the NSSP legal requirements (for example the water monitoring standard and the classification of zones) are not directly applicable in the EU context. Therefore in achieving compliance with the NSSP requirements regarding buffer zones the following clarifications have been agreed with the US FDA:

- US 'approved' areas are considered equivalent to EU 'class A' areas.
- US 'conditionally approved' areas are considered equivalent to EU class A areas with a formal management plan.
- US 'restricted' or 'conditionally restricted' and EU 'class B' or 'class C areas' will not at this time be accepted for reciprocal trade and therefore these designations are not relevant for exports.
- The US FDA requirement for designation of a 'Prohibited' area adjacent to each sewage treatment plant outfall is covered in this annex by designation of a 'buffer zone'. designation, for the purpose of this annex, means that bivalves harvested from the area delineated cannot be exported to the US. It may be acceptable to place such products on the EU market subject to the normal EU classification and regulatory requirements. In this case the Competent Authority should clearly distinguish between these different designations.
- The FDA have clarified that sizing of buffer zones under the NSSP is, in principle, based on calculation of dilution from the faecal indicator count of the impacting discharge(s) to an extent that meets the bacteriological standards set out in the NSSP. Since exports are only agreed for EU class A areas, and this has been agreed as equivalent to US approved areas, the relevant faecal indicator standard to be achieved is 14 faecal coliform MPN per 100ml of water.
- The FDA have confirmed that this dilution is calculated and does not require any laboratory testing. Indeed, laboratory test results are not considered an alternative to the calculation of the necessary dilution. The calculation must be performed and documented.

- For the purposes of buffer zone boundary calculations, *E. coli* concentration can be considered equivalent to faecal coliform concentration, i.e. calculations can be based on either and calculated compliance with 14 *E. coli* per 100ml of water is acceptable.

The recommendations given in Sections A.4.2.2 and A.4.3.2 have been agreed with the FDA.

A4.2 Buffer zones around wastewater discharges

A4.2.1 NSSP Requirement

The US legal requirement for buffer zones around wastewater discharges that the US FDA will audit against is set out in the NSSP MO (FDA, 2013) Section II, Chapter IV .03E(5) as follows:

> *(5) Wastewater Discharges.*
> *(a) An area classified as prohibited shall be established adjacent to each sewage treatment plant outfall or any other point source outfall of public health significance.*
> *(b) The determination of the size of the area to be classified as prohibited adjacent to each outfall shall include the following minimum criteria:*
>
> > *(i) The volume flow rate, location of discharge, performance of the wastewater treatment plant and the bacteriological or viral quality of the effluent;*
> > *(ii) The decay rate of the contaminants of public health significance in the wastewater discharged;*
> > *(iii) The wastewater's dispersion and dilution, and the time of waste transport to the area where shellstock may be harvested; and*
> > *(iv) The location of the shellfish resources, classification of adjacent waters and identifiable landmarks or boundaries.*

Further US guidance on buffer zones is set out in the NSSP MO (FDA, 2013) guidance document, “Sanitary Survey and the Classification of Growing Waters” (Section IV, Chapter II, .04) as follows:

> *The NSSP Model Ordinance also requires that an area in the prohibited classification (closed safety zone) must be established between any sewage treatment plants or other waste discharges of public health significance and any growing area placed in the approved, conditionally approved, restricted, or conditionally restricted classification. The size of the prohibited area should be based on the effectiveness and level of sewage treatment; the location of the shellstock resource that would be affected; the classification of adjacent waters; the total time it would take for the person responsible for the operation of the sewage treatment facility to detect a failure and notify the Authority; the time it would take the Authority to issue a notice to stop shellstock harvesting; and the degree of effluent dilution. Due consideration should be given to the possibility that emergency actions might be necessary on holidays or at night.*

If the buffer zone is sized according to the protection afforded by treated effluent (eg from a sewage treatment plant) then there must also be a formal written ‘management plan’ that demonstrates how, in the case of any discharge of untreated effluent (for example a storm water discharge or emergency overflow associated with the plant), the production area can be closed before any so contaminated products are marketed for export. This plan must be formally agreed between the Competent Authority responsible for the sanitation of the production area (with the authority to close the area) and the authorities responsible for the sewage treatment plant (with access to plant monitoring records etc). The legal requirement is set out in the NSSP MO (FDA, 2013) Section II, Chapter IV @.03 C(2)(a) as follows:

(2) Management Plan Required. For each growing area, a written management plan shall be developed and shall include:

(a) For management plans based on wastewater treatment plant function, performance standards that include:

(i) Peak effluent flow, average flow, and infiltration flow;

(ii) Bacteriological or viral quality of the effluent;

(iii) Physical and chemical quality of the effluent;

(iv) Conditions which cause plant failure;

(v) Plant or collection system bypasses;

(vi) Design, construction, and maintenance to minimize mechanical failure, or overloading;

(vii) Provisions for monitoring and inspecting the waste water treatment plant; and

(viii)Establishment of an area in the prohibited classification adjacent to a wastewater treatment plant outfall in accordance with §E. Prohibited Classification;

(b) For management plans based on pollution sources other than waste water treatment plants:

(i) Performance standards that reliably predict when criteria for conditional classification are met; and

(ii) Discussion and data supporting the performance standards.

A.4.2.2 Recommendations

During the sanitary survey (performed according to Section 2 of this Guide and Section 2 of the Community Guide (European Commission, 2014) human point source discharges that may impact the class A area under consideration for export should be specifically identified in a section of the sanitary survey report.

- Point source human wastewater discharges considered should include: discharges from sewer pipes; storm water discharges; emergency discharges; septic tank discharges.
- Potentially impacting water courses (eg rivers and streams), with human point sources within their catchment, can be considered as a single point source at the position of entry.

For all identified significant human point source discharges identified within the sanitary survey as potentially impacting on the harvesting area, a calculation should be performed of the worst case faecal indicator loading over a 24 hour period taking into account both the concentration of faecal coliforms/*E. coli* present in the effluent and the volume of effluent discharged. The following criteria can be used:

- Either robust analytical measurement of the faecal indicator content of untreated effluent or a standard value of 1.4×10^6 faecal coliforms/*E. coli* per 100ml.
- Actual recorded flow volumes, consented volumes, or the maximum flows possible according to the pipe dimensions

The calculated worst case faecal indicator loading should be compared with the available marine dilution available according to the specific characteristics of the production area to establish the boundary at which a faecal coliform/*E. coli* concentration of 14 per 100ml can be achieved. This can be calculated according to the following options:

- Dilutions can be calculated assuming full mixing with a 24 hour period or;
- Using a hydrodynamic model of the area or;
- According to site-specific dilution dispersion studies (eg using dye release)

- Faecal indicator bacteria decay rates in the marine environment (eg T_{90}) can be factored in if available.

If this boundary is acceptable then the calculations should be documented (against future audit) and the specified buffer zone formally delineated and recorded in the sanitary survey report – particularly noting that it applies only to exports destined for the USA. Note it is a legal requirement of the NSSP that all human point source discharges of significance potentially impacting the area designated for harvest must have a buffer zone established.

If the buffer zone is not acceptable i.e. it is too restrictive on the area desired for commercial harvest, then further calculations taking into account treatment levels (for treated sewer discharges), and the conditions under which intermittent discharges may actually spill, may be performed. In this case it is important to note that the area must have a management plan which closes the area for harvesting when spills occur or when the untreated effluent is discharged thus preventing non-compliant product being sent for export.

In this case:

- Loading calculations are based on robust analytical measurement of faecal indicator content of the treated effluent with boundary calculations performed as described above
- The sewage treatment plant must be fully alarmed such that any treatment failures are immediately notified to the Competent Authority responsible for closure of the harvest area
- Intermittent discharges must be monitored and, when they discharge, be immediately reported to the Competent Authority with authority to close the harvest area
- The management plan should include:
    - A description of the point sources that are controlled under the plan (this should cover all of the significant point sources identified within the sanitary survey as potentially impacting on the harvesting area).
    - A formal agreement between the authorities responsible for these point sources (sewage treatment plant or intermittent discharge) and the Competent Authority with authority to close the production area.
    - The notification procedures for each source.
    - The maximum time delay until notification (including consideration of incidents occurring out of offices hours).
    - The time allowed by the Competent Authority to close the area (prevent harvesting for export).
    - The minimum period between harvest and export product dispatch agreed with the relevant Food Business Operator(s).
    - The procedures to identify any non-complaint product already in the production process.
    - The maximum time delay envisaged in the management plan prior to action by the Competent Authority must not exceed the time agreed with the Food Business Operator for product processing and packaging, i.e. it should not be possible for non-compliant products to be dispatched for export.
- In determining buffer zone boundaries for treated effluents the US FDA have indicated that, in all cases, they would expect a minimum dilution of 1:1000 to be achieved to respect the requirement to consider the virological quality of discharges. Since, in this case, sewage discharges may be in closer proximity to harvested areas it is necessary to more accurately estimate the degree of dilution of the sewage plume in delineating the buffer zone. Buffer zones to achieve at least a 1:1000 dilution can be calculated according to the following options:
    - Dilutions can be achieved assuming full mixing within the volume of water impacted which can be defined by drogue studies or;

- Using a hydrodynamic model of the area or;
- According to site-specific dilution dispersion studies (eg using dye release)

- The management plan should be a formal document, owned by the competent authority, open to audit and preferably placed in the public domain. The buffer zone conditional on the management plan should be explicitly delineated and identified within the management plan. The plan should also specifically record the roles and responsibilities of the various parties necessary for its operation.

Finally, it is agreed with the FDA that there are alternative possible approaches to those described above for calculating the size of the buffer zone necessary to mitigate the virological impacts of a waste water treatment plant. Possibilities include alternative ways of estimating the area impacted by the sewage plume, or verifying the region of virus impact through direct virological analysis of shellfish stocks. Alternative approaches can be used provided that they are documented and based on sound, scientific principles that can be verified.

A.4.3 Requirement for buffer zones around marinas

Boats may discharge untreated effluent and hence marinas are also considered a potential source of faecal pollution requiring a buffer zone. Section 2.13 of this Guide contains a recommendation of a 300 m closure area around harbours and marinas. However, the FDA considers that this criterion is too general for product to be exported to the USA.

A.4.3.1 Requirement

The US legal requirement for buffer zones around marinas that are adjacent to shellfish growing areas is set out in the NSSP MO (FDA, 2013) Section II, Chapter IV @.05 Marinas as follows:

@.05 Marinas.
A. Marina Proper. The area within any marina which is in or adjacent to a shellstock growing area shall be classified as:

(1) Conditionally approved;
(2) Conditionally restricted; or
(3) Prohibited.

B. Adjacent Waters. Waters adjacent to marina waters classified under §A. may be impacted by pollution associated with the marina.

(1) A dilution analysis shall be used to determine if there is any impact to adjacent waters.
(2) The dilution analysis shall be based on the volume of water in the vicinity of the marina.
(3) The dilution analysis shall incorporate the following:
(a) A slip occupancy rate for the marina;
(b) An actual or assumed rate of boats which will discharge untreated waste;
(c) An occupancy per boat rate (i.e., number of persons per boat);
(d) A fecal coliform discharge rate of 2×10^9 fecal coliform per day; and
(e) The assumption that the wastes are completely mixed in the volume of water in and around the marina.
(4) If the dilution analysis predicts a theoretical fecal coliform loading greater than 14 fecal coliform MPN per 100 ml, the waters adjacent to the marina shall be classified as:
(a) Conditionally approved;

(b) Restricted;
(c) Conditionally restricted; or
(d) Prohibited.

(5) If the dilution analyses predicts a theoretical fecal coliform loading less than or equal to 14 fecal coliform MPN per 100 ml, the waters adjacent to the marina may be classified as:

(a) Approved; or
(b) Conditionally approved.

(6) If the Authority chooses not to determine a specific occupancy per boat rate by investigation in specific areas or sites, the Authority shall assume a minimum occupancy rate of two persons per boat.

A.4.3.2 Recommendations

- The approach to calculation of buffer zones sizes for marinas follows the same general principles as set out above for human wastewater inputs.
- Locations within marinas should be deemed as prohibited with respect to product to be exported to the USA. unless there is a defined season when there is no occupation of boats in a marina (see below).
- For adjacent waters, the approach to estimating the buffer zone should be based on the requirements given under items 1, 2 and 3 of Section B of the NSSP section on Marinas (as specified above). The 2 x 10^9 faecal coliform/*E. coli* per day discharge rate applies per person for the total human occupancy rate estimated for the marina.
- As for human wastewater inputs, the required target at the boundary of the buffer zone is a faecal indicator concentration of 14 faecal coliform MPN (or *E. coli*) per 100ml of water.
- As for wastewaters, it is also possible to have a 'conditional' harvesting for marinas and adjacent waters to cover situations where the impact of the marina changes significantly according to known circumstances – for example seasonal use. In this case, as for wastewaters, a management plan should be drawn up which specify the specific circumstances under which harvesting locations within the marina, or relevant the relevant buffer zones, are applicable. If necessary, calculations should be performed, and buffer zone boundaries delineated, for all circumstances specified in the management plan.
- The marina buffer zone(s) should be delineated in a formal document, owned by the competent authority, open to audit and preferably placed in the public domain - particularly noting that they apply only to exports destined for the USA. If applicable, the marina management plan should also be recorded in that document.

A.4.4 References

FDA 2013. National Shellfish Sanitation Program (NSSP): Guide for the Control of Molluscan Shellfish. 2011 Revision. U. S. Department of Health and Human Services, Public Health Service, Food and Drug Administration.

European Commission 2014. Community Guide to the Principles of Good Practice for the Microbiological Classification and Monitoring of Bivalve Mollusc Production and Relaying Areas with regard to Regulation 854/2004. European Commission DG Health and Consumers. Issue 2.