Microbiology Dimension in EU Water Directives

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Microbiology dimension in EU Water Directives

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Summary

At the Johannesburg World Summit on Sustainable Development (2002) the scenario on the world’s water resources was summarised in the following key numbers: 1.1 billion people - 18% of the world’s population - lack access to adequate sanitation. In developing countries, more than 2.2 million people die each year from diseases associated with lack of access to safe drinking water, inadequate sanitation and poor hygiene and a large proportion of people suffer from diseases caused directly or indirectly by the consumption of contaminated water or food or by pathogens that breed in water. However, with adequate supplies of safe drinking water and sanitation, the incidence of some illnesses and death could drop by as much as 75%.

In Europe, although high standards have been reached in some countries and minor supply problems are encountered in all states, outbreaks of waterborne diseases continue to occur. European sanitary measures evaluate faecal pollution for water quality monitoring, through a quantitative analysis of “indicators” (total/faecal coliforms, faecal streptococci) that are not necessarily pathogenic themselves but have the same faecal source of pathogens. Many flaws in the current techniques make this indicator system unreliable. In fact, indicator-based approach is time-consuming, lacks specificity, poorly detects slow growing or Viable But Non-Culturables microorganisms and often misses disease-causing viruses, (Hepatitis A or E, Coxsackie viruses, Adenoviruses), indigenous pathogenic bacteria (Helicobacter and Legionella) as well as protozoan parasites (Cryptosporidium parvum and Giardia). However, up to now, the direct detection of pathogenic organisms is not appropriate for routine monitoring since it is expensive, difficult and, as not all pathogens have been characterised, methods still remain unavailable.
In the framework of a sustainable use of water resources to ensure safe water supply and sanitation protective of human health and the environment, European Union is conducting an extensive range of essentially legislative measures in order to guarantee a shrewd management of water quality and quantity in Europe. Early European water legislation began, in a "first wave", in 1975 and culminated in 2000 with the coming into force of the Water Framework Directive, that represents the decisive step in modernising water management by bringing together the fragmented water-related environmental legislation in order to enhance a coherent implementation.

This report provides an overview of the whole European water-related legislation and extensively focuses on its microbiological aspects. The microbiological parameters to be analysed according to the legislation, the methods suggested and the frequencies of sampling requested are summarised for each directive, so that a comprehensive outlook of microbiology dimension in EU water directives is presented.

**Keywords:** EU Water Directives; indicators; pathogens
Introduction

A sustainable and effective water management, ensuring protection of human health and environment, conservation of water resources and safe water supply and disposal, requires both quality and quantity of water to be considered. In developed countries water consumption has been high in recent decades. This is a consequence of the perception that water is abundant and inexpensive. In many countries, the rising cost of water and the enforcement of national legislation are leading to a reduction in water use and to an increase in investments for water-saving processes and equipment. However, in some cases, rising water prices may lead to the use of alternative water supplies, like private wells, that may be of unacceptable quality (1). There is thus the need of a balance between prices high enough to reduce excessive water demand and acceptable water quality in order to protect consumers health.

The balance of costs and benefits differs according to local conditions and one target of EU programmes to control and prevent water-related diseases should be narrowing the gap between western and eastern European countries. Nevertheless, the western part of Europe still has a significant incidence of diseases which should not be ignored (2, 3). The financial burden on users to pay for water and its sanitation is incredibly low when compared to the health cost incurred by failure to provide safe water to all citizens. Studies indicate that for more severe diseases (hepatitis A, typhoid fever and cholera) costs can reach about 10 - 40 % of gross domestic product per person; for gastroenteritis, a less severe illness, costs are lower, generally 5 % of gross domestic product per person. Moreover, an estimated 60 - 80 % of waterborne diseases could be avoidable by improving water quality and sanitation.

In 1992 World Health Organisation (WHO), United Nations Environment Programme and EU developed the "Protocol on water and health-convention on the protection and use of trans-boundary watercourses and international lakes". In this perspective, EU and United Nation Educational Scientific and Cultural Organisation (UNESCO) are now focusing on the following aims (4):

- to halve the proportion of people who are unable to reach or to afford safe drinking water (DW) by 2015
• to stop the unsustainable exploitation of water resources by developing water management strategies at regional, national and local levels, which promote both equitable access and adequate supplies.

In this framework, at the Johannesburg World Summit on Sustainable Development (WSSD), EU has developed the **Global Water Initiative**. The aim of this multi-stakeholder process is the improvement of the efficiency of existing financing mechanisms through a better coordination. EU will help its partners to develop integrated water resources management plans by 2005 and achieve a sustainable balance between human water needs and those of the environment. All partners are committed to ensure that appropriate priority is given to clean water and sanitation in the future.

In order to ensure safe water supply and sanitation protective of human health and the environment, EU is conducting an extensive range of essentially legislative measures to guarantee a shrewd management of water quality and quantity in Europe. EU water legislation has had, for nearly 30 years, two different approaches: setting water quality standards and laying down emission limit values for specific substances. Recently, in order to ensure the overall consistency of water policy, the fragmented pieces of legislation, covering water of different types and destined for different uses, have been integrated in a “combined approach” by the Water Framework Directive - WFD - (Directive 2000/60/EC of the European Parliament and of the Council).

The “*first wave*” of EU water legislation took place in the latter part of 70’s and culminated in the Drinking Water Directive (CD 80/778/EEC). The pioneer directive, regarding the quality of surface water intended for the abstraction of drinking water (CD 75/440/EEC), was adopted in 1975. Later on, a number of directives based on the approach of regulating the quality of water used for a specific purpose were published: directives concerning the quality of bathing water (CD 76/160/EEC), the quality of fresh waters that need protection or improvement in order to support fish life (CD 78/659/EEC) and the quality of shellfish waters (CD 79/923/EEC). In 1976, the Dangerous Substances Directive (DSD) on pollution caused by certain dangerous compounds discharged into the aquatic environment (CD 76/464/EEC) firstly laid down emission limit values (ELV) for specific substances. However, it only legislated in terms of
basic principles and obligations concerning systems of control; that means it did not include any numerical value. Specific standards were subsequently defined in several daughter directives, issued to implement the DSD.

The "second wave" of EU legislation took place in the early 90's. Legislation to control nitrate pollution in agriculture and pollution by urban wastewater by municipalities was called for. Therefore, in 1991, two directives to supplement the previous water legislation were adopted: CD 91/271/EEC concerning urban wastewater treatment and CD 91/676/EEC for the protection of waters against pollution caused by nitrates from agricultural sources. In 1998, in order to upgrade CD 80/778/EEC to scientific and technological progress, the EU Drinking Water Directive - DWD - (CD 98/83/EC on the quality of water intended for human consumption) was adopted. It provides the framework for drinking water quality standards in EU and it has to be transposed into national legislation by 25.12.2003. WHO guidelines (5) for drinking water quality are used as reference in setting standards. Although EU standards of CD 80/778/EEC have been adopted, many countries still experience problems with certain parameters and are still not fully complying (1). Collating information on compliance with the EU DWD is often difficult, since few countries publish detailed annual reports and data are often presented differently. In Europe, central and eastern countries report the most frequent microbiological compliance problems due to non-continuous supply, intensive industrial activity with no concern to the environment and inadequate water protection (1). Moreover, eastern states experienced many changes in the past decade: ex-USSR legislation had strict standards for DW quality, which were poorly enforced because institutional mechanisms and resources were lacking and methods of analysis were often inadequate. Lack of investment in treatment and distribution facilities also contributed to significant problems. Candidate countries for membership of the EU are preparing or have recently introduced new legislation closely linked to WHO guidelines and/or EU DWD. Many countries still require considerable investment to improve the infrastructure, meanwhile in all MS there is still an incredible lack of implementation due to inconsistencies between legal systems.
EU Water Directives


- Directives containing microbiological parameters
  - Strictly public health related
  - Not strictly public health related

- Directives not containing microbiological parameters

The following Directives (called “daughter” directives) regulate emission limit values and quality objectives for 18 individual substances of the “candidate list I substances” of Directive 76/464/EEC and were amended by Council Directives 90/656/EEC and 91/692/EEC.


Microbiological parameters

Despite the remarkable success of water treatment and sanitation programs in improving public health, sporadic cases and point-source outbreaks of waterborne diseases continue to occur (6). Waterborne pathogens enter human hosts through intact or compromised skin, inhalation, ingestion and direct contact with the mucous membrane of the eye, ear, nose, mouth and genitals. Some opportunistic pathogenic bacteria (*Pseudomonas, Acinetobacter, Serratia, Chromobacterium, Achromobacter, Aeromonas*, etc.) occur naturally in water. Other opportunistic bacteria (*Bacillus, Enterobacter, Klebsiella, Actinomyces, Streptomyces*) are sometimes washed into water from their natural habitat in soil or vegetative matter or may be seeded from re-growth and biofilms in water treatment plants and distribution systems.

Common outdoor recreational activities, such as swimming pool, boating, camping, canoeing and surfing, place humans at risk of waterborne diseases from ingestion or direct contact with contaminated water, giving rise to gastroenteritis, pharingoconjunctivitis, folliculitis, colitis and pneumonia.

In recent years, several so called “new or emerging pathogens” have arisen. They include, on one hand, new recognised pathogens from faecal source such as *Campylobacter jejuni*, pathogenic *Escherichia coli*, *Yersinia enterocolitica*, new enteric viruses (rotaviruses, caliciviruses, Small Round Structure Viruses, astroviruses) and parasites (*Giardia, Cryptosporidium*). On the other hand, some new pathogens are environmental bacteria able to grow in water distribution systems and only recently recognised as relevant pathogens; among these germs should be mentioned *Legionella* spp., *Aeromonas* spp., MAC (*Mycobacterium Avium Complex*) group and *Pseudomonas aeruginosa*.

Pathogenic organisms directly responsible for the spread of diseases are of concern but their detection is difficult, expensive and time-consuming and thus not appropriate for routine monitoring. Moreover, as not all pathogens have been characterised, methods for their detection remain unavailable and, since the presence of pathogens is intermittent and the time of survival in the environment is variable, routine examination of water/wastewater for pathogenic bacteria is not recommended.
For more than 100 years the water industry has depended on methods that detect and enumerate "indicator" organisms for the examination of faecal contamination, potentially correlated with pathogens presence. However, the current water quality assessment requires time-consuming classical culture-based methods involving sample membrane filtration, incubation and biochemical confirmation tests (6).

The ideal indicator should be:

- universally present in the faeces of humans and warm-blooded animals
- not able to grow in natural bodies of water
- easy to detect and enumerate
- persistent and removed during water treatment similarly to waterborne pathogens, so that it acts as indicator of faecal pollution but also monitors the effectiveness of water treatment.

It should be mentioned that these indicators are not necessarily pathogenic themselves but they have the same faecal source as pathogens and, therefore, indicate a faecal contamination of water. However, some pathogens such as *Pseudomonas, Aeromonas, Yersinia, Legionella, Mycobacterium* and *Vibrio* may not correlate with coliform bacteria and traditional indicators may not correlate with viruses and parasites in pristine or ground water. In addition, indicator bacteria may become stressed or injured in water and wastewater and thus are unable to multiply and form colonies under standard laboratory conditions (7) due to structural or metabolic damage (VBNC, Viable But Non-Culturable). As a result, over 90 % of indicator bacteria present in the sample may not be detected resulting in an inaccurate water quality characterisation and leading to the acceptance of a potentially hazardous condition resulting from contamination by resistant pathogens or from the penetration of undetected indicator bacteria through treatment barriers. Moreover, viruses and waterborne pathogens that form cysts are also more resistant to environmental stressors than indicator bacteria.

The EU Directive 80/778/EEC recommends total coliforms, faecal coliforms and faecal streptococci to be evaluated. Differently, the new CD 98/83/EC requires *E. coli* and enterococci to be determined. Indicators are among parameters found most frequently at levels of concern in the drinking water of many European countries (Andorra, Croatia, Czech
Republic, Estonia, Germany, Greece, Iceland, Liechtenstein, Malta, Norway, Slovakia, Slovenia, Turkey and Ukraine) (8).

**Indicators and their characteristics**

**Coliforms**: are Gram-negative, non spore-forming, oxidase-negative, rod-shaped facultative anaerobic bacteria that ferment lactose (with β-galactosidase) to acid and gas within 24 - 48 h at 36 ± 2 °C. *Escherichia, Citrobacter, Enterobacter* and *Klebsiella* but even some environmental genera belong to coliform group. Due to this fact, the role of coliforms as indicator bacteria of faecal pollution has been recently reconsidered and, nowadays, they are principally regarded as indicators of wastewater treatment efficiency. Coliforms belong to the family of *Enterobacteriaceae*, which includes also *Proteus, Serratia, Shigella* and *Yersinia*, that can be potentially pathogenic for humans.

**Thermotolerant coliforms**: are also known as *faecal coliforms*. They produce acid and gas from lactose at 44.5 ± 0.2 °C within 24 ± 2 h. *Escherichia coli*: thermophilic coliform that produces indole from tryptophan, but now also defined as any coliform able to produce β-glucuronidase (although taxonomically up to 10% of environmental *E. coli* may not). It is present at high concentration in the faeces of warm-blooded animals and it is easily culturable. If the ratio *faecal coliforms*/streptococci is 4:1, the contamination is of human source. Presence of total coliforms and absence of faecal coliforms indicate re-growth.

**Faecal streptococci (FS)**: are gram-positive, catalase-negative cocci able to grow on selective media (e.g. azide dextrose broth or m Enterococcus agar); they possess the Lancefield group D antigen and grow on bile aesculin agar at 45 °C.

**Enterococci**: all faecal streptococci that grow at pH 9.6, 10 °C and 45 °C and in 6.5 % NaCl, classified into the genus Enterococcus. They fulfil the following criteria: resistance to 60 °C for 30 min and ability to reduce 0.1 % methylene blue. Enterococci are a subset of faecal streptococci that grow under the conditions outlined above. Alternatively, enterococci can be directly identified as microorganisms capable of aerobic growth at 44 ± 0.5 °C and of hydrolysing 4-methylumbelliferyl-β-D-glucoside (detecting β-glucosidase activity by blue florescence at 366 nm) in the presence of thallium acetate, nalidixic acid and 2,3,5-
triphenyltetrazolium chloride (TTC, which is reduced to red formazan) in the specified medium (ISO/FDIS 7899-1 1998). Due to their resistance to dehydration, they are indicators of (waste)-
water treatment efficiency. Enterococci detection is suggested after maintenance on water
distribution system. Enterococci can be considered indicator of recent faecal pollution and, due
to their resistance to chlorine, indicator of enteroviruses presence.

**Sulphite-reducing clostridia (SRC):** Gram-positive, spore-forming, non-motile, strictly
anaerobic rods that reduce sulphite to H₂S. *Clostridium perfringens:* belongs to SRC, but it
also ferments lactose, sucrose and inositol with gas production. It reduces nitrate, hydrolyses
gelatin and produces lecithinase and acid phosphatase. Not all SRC in receiving waters are
indicators of faecal pollution, hence *C. perfringens* is the appropriate indicator.

**Bifidobacteria:** obligate anaerobic, non-acid-fast, non-spore-forming, non-motile, Gram-
positive bacilli, which are highly pleomorphic and may exhibit branching bulbs (bifids), clubs,
coccoid, coryneform, Y and V forms. They represent one of the most numerous groups of
bacteria in the faeces of warm-blooded animals. They are all catalase-negative and ferment
lactose (except the three insect species *B. asteroides, B. indicum* and *B. coryneforme*).

**Bacteriophages (phages):** are bacterial viruses and are ubiquitous in the environment. For
water quality testing and to model human enteric viruses, somatic coliphages, male-specific
RNA coliphages (F-RNA coliphages) and phages infecting *Bacteroides fragilis* are most
interesting.

**Bacteroides fragilis bacteriophages:** belong to the family *Siphoviridae* with flexible tail
(dsDNA, long non-contractile tails, capsids up to 60 nm) and infect one of the most abundant
bacteria in the gut. Phages to the host strain *B. fragilis* HSP40 are considered to be human-
specific, but phages to *B. fragilis* RYC2056 are more numerous and not human-specific.

**Coliphages:** are somatic coliphages that attack *E. coli* strains via the cell wall; they include
spherical phages of the family *Microviridae* and various tailed phages. The F-RNA coliphages
attack *E. coli* strains via the sex pili (F factor) and are single-stranded RNA non-tailed phages.
Nowadays, the interest in bifidobacteria and bacteriophages as indicators is declining due to the
fact that they do not well correlate with the presence of viruses and parasites in water.
EUROPEAN WATER DIRECTIVES

Water Framework Directive (CD 2000/60/EC)

This directive establishes a framework for Community action in the field of water policy for the protection of inland surface waters, transitional waters, coastal waters and groundwater, in order to prevent and reduce pollution, promote sustainable water use, protect the aquatic environment, improve the status of aquatic ecosystems and mitigate the effects of floods and droughts.

The adoption of the Water Framework Directive (WFD) is the decisive step in bringing together all of the Community water-related environmental legislation, emphasising that all the water directives have to be implemented coherently.

Community environment policy has evolved towards an emphasis on the role of science and informed participation in meeting environmental goals, based on the experience of implementing existing legislation. Moreover, rapid developments in science and technologies make it compulsory to maintain, revise or upgrade the existing water directives. There is also the need to rationalise and optimise the implementation of water quality management through various steps, including reducing the number of parameters to be monitored, and introducing new tools and more robust parameters. All these objectives are linked to sustainable development, resources protection and water security. MS and Candidate Countries (CC) have to achieve a common agreement on cooperation between MS and the Commission. At the same time it is of crucial importance the involvement of stakeholders and non-governmental organisations (NGOs) in implementing the Directive.

Main objectives of WFD are:

- expanding water protection to all the waters, ground-waters (GW) and surface waters (SW) including coastal waters
achieving "good status" for these waters within a deadline of 15 years, with a proper ecological dimension. For GW, good status is measured in terms of quantity and chemical purity. For SW, ecological and chemical quality are the criteria.

- **integrated river basin management** across administrative and political borders, with coordinated programs of measures.
- Emissions and discharges controlled by a "combined approach" of emission limit values and quality standards, plus an obligation to phase out particular hazardous substances.
- Introducing *water pricing policies*, which result in an incentive to use in a sustainable way and to protect resources.
- Getting the *citizen involved* more closely by strengthening public participation.

### Directives including microbiological parameters

**Drinking Water Directive (CD 80/778/EEC) relating to the quality of water intended for human consumption**

This directive lays down at Community level minimum quality and control standards for *water intended for human consumption* and for food production. For the first time *health related criteria* for drinking water are established. It has been revised by the Drinking Water Directive 98/83/EC.

*Water for human consumption* is all water used for this purpose, either in its original state or after treatment, regardless of origin, whether supplied for consumption, or whether used in a food production undertaking for the manufacture, processing, preservation or marketing of products or substances intended for human consumption and affecting the wholesomeness of the foodstuff in its finished form.
The Directive does not concern:

- waters for medicinal products (CD 65/65/EEC; CD 93/39/EEC)
- Natural Mineral Water.

A total of 62 checking parameters are listed in Annex I of the directive (tables A, B, C, D, E) and can be grouped into six categories:

1. organoleptic quality (colour, odour, taste...)
2. physico-chemical parameters (temperature, pH...)
3. substances undesirable in high amounts (nitrates...)
4. toxic substances (mercury, pesticides...)
5. microbial contaminants (faecal coliforms...)
6. minimum required concentration (hardness, alkalinity...).

MS shall fix values for these parameters. Values must be less than or the same as the values shown in the MAC (Maximum Admissible Concentration) column in the directive, taking as basis the value appearing in the GL (Guide Level) column.

MS may refrain from fixing values for which no value is reported, as long as these values have not been determined by the Council.

Criteria for microbiological analysis at source

Water intended for human consumption should require:

- absence of parasites and pathogenic microorganisms
- quantitative determination of revivable total colony count indicative of faecal contamination.

Microbiological parameters specified in the Annex I of the directive are listed in tables 1 and 2.
### Table 1, 2. Microbiological parameters (CD 80/778/EEC, Annex I, E)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample volume (ml)</th>
<th>Guide level</th>
<th>Maximum admissible concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Membrane filtration method</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Multiple tubes method</td>
</tr>
<tr>
<td>Total coliforms</td>
<td>100</td>
<td>-</td>
<td>0 MPN&lt;1</td>
</tr>
<tr>
<td>Faecal coliforms</td>
<td>100</td>
<td>-</td>
<td>0 MPN&lt;1</td>
</tr>
<tr>
<td>Faecal streptococci</td>
<td>100</td>
<td>-</td>
<td>0 MPN&lt;1</td>
</tr>
<tr>
<td>Sulphite-reducing Clostridia</td>
<td>20</td>
<td>-</td>
<td>0 MPN&lt;1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample volume (ml)</th>
<th>Guide level (GL)</th>
<th>Maximum admissible concentration (MAC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bacteria counts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>for waters supplied for human consumption</td>
<td>37 °C</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>22 °C</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Total bacteria counts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>for water in closed containers</td>
<td>37 °C</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>22 °C</td>
<td>1</td>
<td>20</td>
</tr>
</tbody>
</table>

*If it is necessary* (but it is not compulsory) samples of water intended for human consumption should be *examined also for* the following pathogens:

- *Salmonella*
- Pathogenic staphylococci
- Faecal bacteriophages
- Enteroviruses

Moreover, such water *should not contain*:

- Parasites
- Algae
- Other organisms such as animalcules

Microbiological analyses are conducted according to scientific methods laid down in the Annex III of the directive (table 3). However, other methods can be used providing that results are *equivalent to or comparable* with those obtained by the methods above mentioned.
Table 3. Methods of analysis for microbiological parameters (CD 80/778/EEC, Annex III)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliforms (*)</td>
<td>Fermentation in multiple tubes. Subculturing of the positive tubes on a confirmation medium. Count according to Most Probable Number or Membrane filtration and culture on an appropriate medium such as Tergitol lactose agar, endo agar, 0.4 % Teepol broth, sub-culturing and identification of the suspect colonies</td>
</tr>
<tr>
<td>Faecal coliforms (*)</td>
<td>Incubation temperature for total coliforms: 37 °C Incubation temperature for faecal coliforms: 44 °C</td>
</tr>
<tr>
<td>Faecal streptococci (*)</td>
<td>Sodium azide (Litsky). Count according to MPN Membrane filtration and culture on an appropriate medium A spore count, after heating the sample to 80 °C by: • Seeding in a medium with glucose, sulphite and iron, counting the black-halo colonies • Membrane filtration, deposition of the inverted filter on a medium with glucose, sulphite and iron covered with agar, count of black colonies • Distribution in tubes of differential reinforced clostridial medium (DRCM), sub-culturing of the black tubes in a medium of litmus-treated milk, count according to MPN</td>
</tr>
<tr>
<td>Total counts (*)</td>
<td>Inoculation by placing in nutritive agar</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Concentration by membrane filtration. Inoculation on a pre-enriched medium. Enrichment, sub-culturing on isolating agar. Identification</td>
</tr>
<tr>
<td>Pathogenic staphylococci</td>
<td>Membrane filtration and culture on a specific medium (e.g. Chapman’s hypersaline medium). Test for pathogenic characteristics</td>
</tr>
<tr>
<td>Faecal bacteriophages</td>
<td>Guelin’s process</td>
</tr>
<tr>
<td>Enteroviruses</td>
<td>Concentration by filtration, flocculation or centrifuging, and identification</td>
</tr>
<tr>
<td>Protozoa</td>
<td>Concentration by filtration on a membrane, microscopic examination, test for pathogenicity</td>
</tr>
<tr>
<td>Animalcules (worms - larvae)</td>
<td>Concentration by filtration on a membrane. Microscopic examination. Test for pathogenicity</td>
</tr>
</tbody>
</table>

(*) incubation period is generally 24 or 48 hours except for total counts, when it is of 48 or 72 hours.
Drinking Water Directive (CD 98/83/EC) on the quality of water intended for human consumption

Scientific and technological progress has made it necessary to adapt the CD 80/778/EEC. The most important change is that drinking water quality should comply at the consumers tap. The compliance is focused on essential quality and health parameters, leaving MS free to ensure additional monitoring on a case-by-case basis (article 6). By consequence, MS are required to set values for additional parameters not included in the directive or adopt more stringent standards, if necessary, to protect human health within their territories or for ensuring the production, distribution and inspection of water.

Objective: protect human health from the adverse effects of any contamination of water intended for human consumption by ensuring that it is wholesome and clean; that means it is free from microorganisms and parasites and it meets the minimum requirements of Annex I (part A and B) of the directive.

Waters intended for human consumption falling under the DWD:

- treated or untreated water for drinking, cooking, food preparation and other domestic purposes, regardless its origin and whether it is supplied
- water for production of food and of products intended for human consumption
- domestic distribution system (pipes, fittings and appliances installed between the tap and the distribution network) unless they are under the responsibility of the water supplier.

Waters intended for human consumption not falling under the DWD:

- Natural Mineral Waters (CD 80/777/EEC; CD 96/70/EC)

MS may exempt from the provision of the directive:

- water for purpose for which the authorities are satisfied that it has no influence on the health of the consumer
- water for human consumption providing less than 10 m³/d unless it is supplied as part of a commercial/public activity.
Quality parameters listed in Annex I of the directive are divided into:

- microbiological parameters (Part A)
- chemical parameters (Part B)
- indicator parameters (Part C).

The values set for the above-mentioned parameters in Annex I shall not be less stringent. Values for Part C parameters need to be fixed only for monitoring purposes.

*Parametric values* for substances shall be complied with in case of: a) water emerging from the tap in case of water from a distribution network, b) water emerging from the tanker in case of water supplied from a tanker, c) water at the point at which it is put into bottles, d) water at the point at which, in food production, is used in the undertaking. Standard values, laid down in Annex I of CD 98/83/EC, are based on scientific knowledge available, on public health consideration, on risk assessment and refer to the World Health Organisation "Guidelines for DW quality".

MS should establish *monitoring* programs appropriate to local needs to check that water meets the requirements of the directive. Monitoring samples should be taken so that they are representative of the quality of the water consumed throughout the year. Any contamination from disinfection by-products should be kept as low as possible without compromising the disinfection. The competent authorities shall determine sampling points and meet the requirements set in Annex II. *Methods of analysis other* than those specified in Annex III of the directive *may be used*, providing it can be demonstrated that the results obtained are at least as reliable as those produced by the methods specified.

In the event of non-compliance, MS should investigate the cause and ensure that the necessary remedial action is taken as soon as possible and measures taken should not deteriorate the present quality of water.

MS shall ensure that *no substances or materials* for any new installation used for preparation or distribution of water or impurities associated with such substances or materials *remain in water* in concentration higher than is necessary for the purpose of their use.
In order to *adequately inform the consumers*, each MS shall publish a report every 3 years on the quality of water with the objective of informing consumers.

Timescale for compliance is within 5 years of directive entry into force.

Derogation, in exceptional circumstances and for geographically defined areas, shall be limited to as short time as possible and shall not exceed three years.

Review of Annexes should be at least every 5 years in response to scientific and technical progress.

*Criteria for microbiological analysis*

Microbiological parameters and methods of analysis suggested by the directive are reported in tables 4 and 5. Methods are given whenever CEN/ISO methods are available, pending the possible future adoption (article 12) of further CEN/ISO international methods for these parameters. MS may use alternative methods, providing that the obtained results are at least as reliable as those produced by the methods specified. For *Clostridium perfringens* (including spores) the following method is suggested: membrane filtration + anaerobic incubation on m-CP agar at 44 °C for 21 h followed by counting of opaque yellow colonies that turn to pink or red after exposure to ammonia vapours for 20 - 30 minutes.


<table>
<thead>
<tr>
<th>Parameter</th>
<th>Methods</th>
<th>Parametric value</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococci (microbiology)</td>
<td>ISO 7899-2</td>
<td>0/100 ml</td>
<td>0/250ml for water in bottle</td>
</tr>
<tr>
<td><em>E. coli</em> (microbiology)</td>
<td>ISO 9308-1</td>
<td>0/100 ml</td>
<td>0/250ml for water in bottle</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>EN/ISO 12780</td>
<td>0/250 ml</td>
<td>Only for water to be sold in bottle</td>
</tr>
<tr>
<td>total count 37°C (indicator)</td>
<td>EN/ISO 6222</td>
<td>20/ml</td>
<td>Only for water to be sold in bottle</td>
</tr>
<tr>
<td>total count 22°C (indicator)</td>
<td>EN/ISO 6222</td>
<td>100/ml</td>
<td>Only for water to be sold in bottle</td>
</tr>
</tbody>
</table>
Tab 5. Indicator parameters (CD 98/83/EC, Annex I-Part C, Annex III)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ISO</th>
<th>Parametric value</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliform</td>
<td>9308-1</td>
<td>0/100ml</td>
<td>Number/250ml for bottled water</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em> (including spores)</td>
<td>-</td>
<td>0/100ml</td>
<td>To be measured only if water originates from or is influenced by surface water. In case of non compliance, MS must investigate the supply</td>
</tr>
<tr>
<td>Total count 22 °C and 37 °C</td>
<td>EN/ISO 6222</td>
<td>No abnormal change</td>
<td>Necessary only for water offered for sale in bottles</td>
</tr>
</tbody>
</table>

As stated in article 7, regular monitoring of the quality of water should be carried out with the following specifications:

**Check monitoring**: its purpose is to regularly provide information on the organoleptic and microbiological quality of water and on the effectiveness of drinking water treatment (disinfection) in order to determine whether or not water complies with the relevant parametric values laid down in this directive. From the microbiological point of view, parameters reported in table 6 must be subject to check monitoring:


<table>
<thead>
<tr>
<th>Parameter</th>
<th>Methods</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>ISO 9308-1</td>
<td></td>
</tr>
<tr>
<td><em>Clostridium perfringens</em> (including spores)</td>
<td>-</td>
<td>To be measured only if water originates from or is influenced by surface water</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>EN/ISO 12780</td>
<td>Necessary only for water offered for sale in bottles</td>
</tr>
<tr>
<td>Total count 22 °C and 37 °C</td>
<td>EN/ISO 6222</td>
<td>Necessary only for water offered for sale in bottles</td>
</tr>
<tr>
<td>Coliform bacteria</td>
<td>ISO 9308-1</td>
<td></td>
</tr>
</tbody>
</table>

**Audit monitoring**: provides information necessary to determine whether or not all of the directive’s parametric values are being complied with.
MS shall ensure **additional monitoring** on a case-by-case basis of microorganisms for which no parametric value has been set if there is reason to suspect that they may be present. In Italy, for example, CD 98/83/EC has been taken into account by the Decreto Legislativo 2 Febbraio 2001, n. 31 and its integration and modification (Decreto Legislativo 2 Febbraio 2002, n. 27), which establish that it is up to the competent authorities the decision to investigate the following additional parameters: algae, anti *E. coli* bacteriophages, fungi, nematodes, pathogenic enterobacteria, enteroviruses, protozoa, *Pseudomonas aeruginosa*, and pathogen staphylococci. The ISS (Istituto Superiore di Sanità) is in charge of dissemination of standard protocols for the investigation of these parameters. The legislation establishes that anti *E. coli* bacteriophages, enteroviruses, pathogen enterobacteria and pathogenic staphylococci should be absent in water intended for human consumption.

Minimum frequencies of sampling (Annex II) are reported in tables 7 and 8, respectively for human consumption and bottling.

**Tab 7. Minimum frequency of sampling and analysis for water intended for human consumption supplied from a distribution network or from a tanker or used in food production undertaking (CD 98/83/EC, Annex II, table B1)**

<table>
<thead>
<tr>
<th>Volume of water produced or distributed within a supply zone each day - m³</th>
<th>Check monitoring number of samples per year</th>
<th>Audit monitoring number of samples per year</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100</td>
<td>To be decided by MS</td>
<td>To be decided by MS</td>
</tr>
<tr>
<td>&gt;100</td>
<td>&lt;1000</td>
<td>4</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>≤10000</td>
<td>4 + 3 for each 1000 m³/d and part thereof of total volume</td>
</tr>
<tr>
<td>&gt;10000</td>
<td>≤100000</td>
<td>4 + 3 for each 1000 m³/d and part thereof of total volume</td>
</tr>
<tr>
<td>&gt;100000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Tab 8. Minimum frequency of sampling and analysis for water put into bottles or containers intended for sale (CD 98/83/EC, Annex II, table B2)

<table>
<thead>
<tr>
<th>Volume of water produced for offering for sale in bottles or containers each day -m³-</th>
<th>Check monitoring number of samples per year</th>
<th>Audit monitoring number of samples per year</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&gt;10</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>&gt;60</td>
<td>1 for each 5 m³ and part thereof the total volume</td>
<td>1 for each 100 m³ and part thereof the total volume</td>
</tr>
</tbody>
</table>

Natural Mineral Waters Directive (CD 80/777/EEC)

Objective: to harmonise the laws of MS relating to the exploitation and marketing of natural mineral waters in order to facilitate their movement within the Community.

In 1996 it has been amended by directive 96/70/EC of the European Parliament and of the Council.

Natural Mineral Water is defined as microbiologically wholesome water, originating in an underground water table or deposit (protected from pollution) and emerging from a spring tapped at one or more natural or bore exits. It can be distinguished from ordinary drinking water due to its nature, which is characterised by its mineral content, trace elements or other constituents and due to its original state (CD 80/777/EEC, Annex I).

The directive defines characteristics of natural mineral waters, treatments and additions that may be made and condition of exploitation of springs.

The directive concerns:

- waters extracted from the ground and recognised by the responsible authorities as Natural Mineral Water satisfying specific criteria.
- waters extracted from the ground of a third country and imported into EC and recognised by the responsible authorities as Natural Mineral Water.
The Directive shall not apply to:

- Natural Mineral Waters used at source for curative purposes in thermal or hydro-mineral establishments
- waters for medicinal products (CD 65/65/EEC; CD 93/39/EEC)
- Natural Mineral Waters intended for export to third countries.

The allowed treatments to which Natural Mineral Water, in its state at source, may be subjected, are specified: CO₂ can be introduced/reintroduced and any disinfection, addition of bacteriostatic elements or any other treatment likely to change the viable colony count, shall be prohibited.

The revivable total colony count of a Natural Mineral Water at source shall conform to its normal viable colony count and give satisfactory evidence of the protection of the source against all contamination. After bottling the total colony count at source may not exceed 100/ml at 20 - 22 °C in 72 h on agar-agar or agar-gelatin mixture and 20/ml at 37 °C in 24 h on agar-agar. Total colony count shall be measured within 12 h following bottling, the water being maintained at 4 °C.

At source and during its marketing Natural Mineral Waters should be free from:

- Parasites and pathogenic microorganisms
- *E. coli*, coliforms and faecal streptococci /250 ml sample
- Sporulated sulphite reducing anaerobes /50 ml sample
- *Pseudomonas aeruginosa* /250 ml sample.

At the marketing stage: the revivable total colony count of Natural Mineral Water may only be that resulting from the normal increase in the bacteria content which it had at source.

All indications attributing to Natural Mineral Water properties relating to the prevention, treatment or cure of a human illness shall be prohibited. MS may authorise the indication “stimulates digestion” or “may facilitate hepato-biliary functions”.

The directive classifies Effervescent Mineral Water into three categories according to its content in carbon dioxide.
Criteria for microbiological analysis at source

- absence of parasites and pathogenic microorganisms
- quantitative determination of revivable total colony count indicative of faecal contamination.

Table 9. Microbiological parameters (CD 80/777/EEC, Annex I)

<table>
<thead>
<tr>
<th>Microbiological parameter</th>
<th>Sample volume</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total colony count 22 °C</td>
<td>1 ml</td>
<td>20 UCF/1 ml at source</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 UCF/1 ml after bottling</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10⁴ UCF/1 ml at marketing stage</td>
</tr>
<tr>
<td>Total colony count 37 °C</td>
<td>1 ml</td>
<td>5 UCF/1 ml at source</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 UCF/1 ml after bottling</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10⁴ UCF/1 ml at marketing stage</td>
</tr>
<tr>
<td>Total coliforms</td>
<td>250 ml</td>
<td>0 UCF/250 ml</td>
</tr>
<tr>
<td>Faecal coliforms</td>
<td>250 ml</td>
<td>0 UCF/250 ml</td>
</tr>
<tr>
<td>Faecal streptococci</td>
<td>250 ml</td>
<td>0 UCF/250 ml</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>250 ml</td>
<td>0 UCF/250 ml</td>
</tr>
<tr>
<td>Sporulated sulphite reducing anaerobes</td>
<td>50 ml</td>
<td>0 UCF/50 ml</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>250 ml</td>
<td>0 UCF/250 ml</td>
</tr>
</tbody>
</table>

Bathing Water Directive (CD 76/160/EEC)

This directive sets quality requirements to “reduce the pollution of bathing water and to protect such water against further deterioration”. Standards are set in order “to protect environment and public health”.

Bathing water is defined as all running or still fresh waters (or parts thereof) and marine waters in which bathing is explicitly authorised by competent authorities or is not prohibited and is traditionally practised by a large number of bathers. A bathing area is any place where bathing
water is found. A bathing season is the period during which a large number of bathers are expected.

The directive concerns all bathing waters with the exception of water intended for therapeutic purposes, waters used in swimming pools and waters for medicinal products (CD 65/65/EEC; CD 93/39/EEC).

Physical, chemical and microbiological parameters, their limit values, guide values and the minimum frequency of sampling and analysis are indicated. MS should set, for all or each individual bathing area, the values for parameters given in Annex I of CD 76/160/EEC. For parameters for which no values are given in Annex I, MS may decide not to fix any values; values set may not be less stringent than those given in column I of the Annex. Where values appear in column G of the Annex, whether or not there is a corresponding value in column I, MS shall endeavour to observe them as guidelines.

Water samples conform to parametric values in the case of:

- 95 % of the samples for parameters corresponding to those specified in column I
- 90 % of the samples in all other cases with the exception of “total coliform” and “faecal coliform” where the percentage may be 80 % and if, in the case of the 5, 10 or 20 % of the samples which do not comply
- water does not deviate from the parametric values in question by more than 50 %, except for microbiological parameters, pH and dissolved oxygen
- consecutive water samples taken at statistically suitable intervals do not deviate from the relevant parametric values.

**Criteria for microbiological analysis**

Reference methods of analysis for the parameters concerned are reported in table 10. Laboratories employing other methods must ensure that the results obtained are equivalent or comparable to those specified.
Tab 10. Microbiological requirements for bathing water (CD 76/160/EEC)

<table>
<thead>
<tr>
<th>Microbiological parameters</th>
<th>G</th>
<th>I</th>
<th>Minimum sampling frequency</th>
<th>Method of analysis and inspection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliforms/100 ml</td>
<td>500</td>
<td>10 000</td>
<td>Fortnightly (1)</td>
<td>Fermentation in multiple tubes. Sub-culturing of the positive tubes on a confirmation medium. Count according to MPN (most probable number) or membrane filtration and culture on an appropriate medium such as Tergitol lactose agar, endo-agar, 0.4% Teepol broth, sub-culturing and identification of the suspect colonies. In the case of (1) and (2), the incubation temperature is variable according to whether total or faecal coliforms are being investigated.</td>
</tr>
<tr>
<td>Faecal coliforms/100 ml</td>
<td>100</td>
<td>2 000</td>
<td>(2)</td>
<td>Litsky method. Count according to MPN (most probable number) or filtration on membrane. Culture on an appropriate medium.</td>
</tr>
<tr>
<td>Faecal streptococci/100 ml</td>
<td>100</td>
<td>-</td>
<td>(2)</td>
<td>Concentration by membrane filtration. Inoculation on a standard medium. Enrichment – sub-culturing on isolating agar - identification.</td>
</tr>
<tr>
<td>Salmonella/litre</td>
<td>-</td>
<td>0</td>
<td>(2)</td>
<td>Concentrating by filtration flocculation or centrifuging and confirmation.</td>
</tr>
<tr>
<td>Enteroviruses PFU/10 litres</td>
<td>-</td>
<td>0</td>
<td>(2)</td>
<td></td>
</tr>
</tbody>
</table>

G= guide; I= mandatory

(1) when a sample taken in previous years produced results which are appreciably better than those in Annex I and when no new factor likely to lower the quality of the water has appeared, the competent authorities may reduce the sampling frequency by a factor of 2.

(2) concentration to be checked by the competent authorities when an inspection in the bathing area shows that the substance may be present or that the quality of water has deteriorated.

The current Bathing Water Directive is now more than 25 years old and, because of the improvement in science and technology, it is now overdue for revision and update. There is the need to rationalise and optimise the implementation of the bathing water quality management through reducing the number of parameters to be monitored, introducing new tools and more robust parameters.

The objective of the Communication is to highlight the strengths and difficulties of bathing water management and present possible approaches for the new directive, based on experience on this area.

Main criticisms to the Council Directive 76/160/EEC on bathing water are:

- some parameters are outdated and others are no longer relevant (mandatory and guidelines microbiological standards were published before many of the major epidemiological studies had been carried out)
- monitoring is done only for compliance checking and not in order to gain a better understanding of the bathing waters
- the directive does not specify analysis methods, so laboratories use a variety of methods and results are not fully comparable
- microbiological analysis requires considerable time (24 – 48 h) which means that, in case the water sample is confirmed to be non compliant, any (re)action to address that non compliance will be too late and people might have been exposed to pollution.

Water quality standards are indispensable. They have to be ambitious and legally binding. A zero-risk cannot be guaranteed and the possibility of failures or accidents strengthens the argument for ambitious standards. By minimising the regular impact of human activity on bathing water quality and lowering the "normal" level of contaminants in a bathing area as much as possible, the impacts of an unexpected pollution event can be reduced. It is necessary to look beyond what is happening at, or in the direct vicinity of a bathing area to take into account the hinterland in terms of land use, discharges upstream, etc. It is also becoming more than ever necessary to have good quality information in near real-time about the bathing area.
Main tasks of the new Bathing Water Directive are supposed to be monitoring water quality and actively tackling pollution sources.

Identification of bathing areas

Under Directive CD 76/160/EEC there is no definition for “bathing” and ‘bathing area/water”, leaving too much room for interpretation. The new directive would correct this by introducing clear and unambiguous definitions. The main use of bathing water is for recreation and tourism. The new directive would concern the quality of bathing waters, with the exception of water intended for therapeutic purposes, water used in swimming pools and of confined waters that are subject to chemical disinfection. Bathing means direct body contact with water involving head submersion and/or risk of ingestion of water. Bathing water includes all running and still inland surface waters, transitional waters and coastal waters actively promoted for bathing and/or regularly used for bathing. Directive CD 76/160/EEC does not foresee mechanisms for identification of bathing areas.

Compliance

One weak point in an excessive emphasis on monitoring i.e. for areas to be in compliance, the only requirement is that a defined proportion of water samples have to meet the mandatory standard.

In the new directive there will be requirements for compliance with the quality standards and also for reaction when these standards are breached (water quality management). There should be formal obligations for immediate action during the season to respond to occasional non-compliance, as well as for long-term action in case of “structural” non-compliance.

Survey, monitoring and trends in water quality

CD 76/160/EEC obligates MS to monitor bathing water during the bathing season. Currently the quality status of the beach is calculated solely on the basis of the number of samples that either fail or pass the standards and gives no additional information. The new directive should develop a beach profile describing, quantifying, understanding and mapping all potential sources of pollution or contamination on and in the vicinity of the bathing area. This information could be useful for long-term preservation or improvement programs. However, a one-time survey is not sufficient for bathing water management. Continued monitoring of water
quality is necessary and monitoring programs should be designed to ensure the most effective use of sampling resources.

**Standard setting and methods of analysis**

CD 76/160/EEC contains both microbiological (public health) and physico-chemical (environmental/ecological) parameters. As the WFD will specifically focus on the ecological aspects of water bodies, the new Bathing Water Directive should focus on health standards. WHO recommendations can be used as the scientific starting point in the development of the Community standards, taking into account public health protection and a realistic cost/benefit relationship. WHO proposes *Intestinal Enterococci* as the best indicator for microbiological contamination of coastal waters; the Commission would propose in addition *Escherichia coli* as an indicator for microbiological contamination of fresh water bathing areas.

To overcome the long time required by microbiological analysis, the Commission would propose two “instant” indicators of contamination: divergence from normal pH and/or turbidity for fresh waters and changes in “normal” salinity for coastal waters. Algae and macrophyte blooming should be also evaluated, as they are closely connected to nutrients increase (e.g. due to human faecal contamination) in water.


The overall purpose of the new directive is to preserve, protect and improve the quality of the environment and to protect human health.

Although the Bathing Water Directive has a distinct contribution to the integration of policies on the environment and tourism and a distinct identity, it should be closely coordinated with the WFD. Moreover, mass growth of toxic algae and/or macrophyte is increasingly becoming a problem. There is a strong correlation between these phenomena and high levels of nutrients, which are caused primarily by human activity and can thus be controlled or influenced. The new directive should contain some sort of protocol setting out what to do when algae or macrophyte blooms occur coordinated with the Urban Wastewater Treatment Directive (CD 91/271/EEC) and the Directive on Nitrates (CD 91/686/EEC).
The Commission proposes a reduction from 19 parameters to 2 key microbiological parameters (Intestinal enterococci and E. coli), complemented by visual inspection (algae bloom, oil) and pH measurements in fresh waters. In CD 76/160/EEC, three microbiological parameters were monitored (total Coliforms, faecal Coliforms and faecal Streptococci) but, the first two belong to the same family of bacteria and the third is only taken into account as a guide to better water quality. The Scientific Committee on Toxicity, Ecotoxicity and the Environment (SCTEE), based on scientific and epidemiological studies, stated that: a) Intestinal Enterococci and E. coli are representative of most reported episodes and correlate with health problems; b) research on viral indicators remain necessary; c) to avoid inter-laboratories difference, the Commission advocates linking one single (ISO CEN) method to each parameter. This proposal will provide for considerable reduction of costs, avoid parallel efforts, but at the same time not entail any reduction in the level of protection of the citizens.

The directive shall cover all bathing waters except water for therapeutic use, water in swimming pools and spa pools, confined water subjected to treatment and waters in confined surface waters artificially created and separated from natural waters like groundwater, surface or coastal waters.

MS shall ensure all bathing waters respect good quality status and shall promote the achievements of quality standards that conform to those set out for excellence quality. As a result of the yearly assessment of the sets of water quality data, MS shall classify the water quality of bathing water as poor (subjected to a thorough study at least once a year), good (subjected to bi-annual analysis), and excellent (subjected to tri-annual analysis).

The directive shall propose harmonised standards for treatment of samples including handling, analysis, storage and transport in order to reduce the risks of contamination (Annex V of the Proposal) and the values reported are based upon 95 percentile evaluation as described in Annex I of the Proposal.
<table>
<thead>
<tr>
<th>Microbiological parameters</th>
<th>Excellent quality (guide)</th>
<th>Good quality (obligatory)</th>
<th>Methods of analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal enterococci in cfu/100 ml</td>
<td>100</td>
<td>200</td>
<td>ISO 7899</td>
</tr>
<tr>
<td>Escherichia coli in cfu/100 ml</td>
<td>250</td>
<td>500</td>
<td>ISO 9308</td>
</tr>
<tr>
<td>Phytoplankton blooms or macro-algae proliferation (*)</td>
<td>Negative result on tests</td>
<td></td>
<td>Microscopic monitoring (**)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>toxicity tests (***)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>and visual inspection</td>
</tr>
</tbody>
</table>

(*) only for sites revealed physically sensitive to specific toxic blooms
(*** determination and counting of cells
(****) mouse test, skin test or direct toxin dosage in plankton cells or water

MS are requested to give the public all the necessary information to allow people to make their own choice about where and if to go bathing. Information should be available at all times at the bathing zone and appropriate media and technologies shall be used to actively disseminate it.

**Surface Water intended for the abstraction of drinking water Directive (CD 75/440/EEC)**

The directive lays down quality requirements which surface fresh water used or intended for use in abstraction of drinking water must meet after application of appropriate treatment.

*Drinking water: all surface waters intended for human consumption and supplied by distribution networks for public use.* Groundwater, brackish water intended to replenish and water-bearing beds are not included.

Minimum quality requirements to be met by surface fresh water are set as:
- physical, chemical and microbiological parameters and the appropriate limit and guide values
- minimum frequency of sampling and analysis
- common non-mandatory reference methods for measuring the parameters.

In Annex I of the directive, waters are classified into three categories (A1, A2, A3) on the basis of their physical, chemical and microbiological characteristics (46 parameters, Annex II of the directive) and a method of treatment is defined for each category (Annex I). Surface waters having characteristics falling short of the mandatory limiting values corresponding to treatment type A3 may not be used for the abstraction of drinking water.

MS set values for parameters and frequency of analysis in accordance with guidelines in the directive. The values may not be less stringent than those given in column “I” (mandatory, “Imperative” value) of Annex II of the directive. Where values appear in the “G” column, MS shall endeavour respect them as guidelines. If no values for parameters are set, MS are under no obligation to set one. On the other hand, MS may set more stringent requirements. Surface water is assumed to conform to the parameters if 95 % of samples taken at regular time intervals at the same sampling point, meet the Annex II limit values (90 % in the other cases).

According to CD 75/440/EEC, responsible authorities of MS are required to define frequency of sampling and methods of analysis. However, since CD 79/869/EEC was put into force, MS should, as far as possible, use reference methods and frequency of sampling and analysis herein reported.

**Tab 12. Definition of the standard methods of treatment for transforming surface water of categories A1, A2, A3 into drinking water (CD 75/440/EEC, Annex I)**

<table>
<thead>
<tr>
<th>Category</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Simple physical treatment and disinfection</td>
</tr>
<tr>
<td>A2</td>
<td>Normal physical treatment, chemical treatment and disinfection (pre-chlorination, coagulation, flocculation, decantation, filtration, disinfection)</td>
</tr>
<tr>
<td>A3</td>
<td>Intensive physical and chemical treatment, extended treatment and disinfection (chlorination to break-point, coagulation, flocculation, decantation, filtration, adsorption, disinfection)</td>
</tr>
</tbody>
</table>
Tab 13. Microbiological parameters (CD 75/440/EEC, Annex II)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>A1 G</th>
<th>A1 I</th>
<th>A2 G</th>
<th>A2 I</th>
<th>A3 G</th>
<th>A3 I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliforms 37 °C</td>
<td>/100 ml</td>
<td>50</td>
<td>5000</td>
<td>5000</td>
<td>50000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faecal coliforms</td>
<td>/100 ml</td>
<td>20</td>
<td>2000</td>
<td>2000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faecal streptococci</td>
<td>/100 ml</td>
<td>20</td>
<td>1000</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not present in 5000 ml</td>
<td></td>
<td>Not present in 1000 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Methods of measurement/sampling/analysis of surface drinking water Directive (CD 79/869/EEC)

This directive concerns the reference methods of measurement (Annex I of the directive) and frequencies of sampling and analysis (Annex II) for the parameters listed in directive CD 75/440/EEC.

MS shall, as far as possible, use the reference methods of measurement reported in Annex I; the limit of detection (defined as the minimum value of the parameter examined which is possible to detect) must be respected. For chemical and physical parameters even precision and accuracy of the methods must be respected.

MS shall fix the frequencies of sampling and analysis for each parameter. Frequencies shall not be less than the minimum annual frequencies of sampling and analysis recommended.

The containers used for samples, the agents or methods used to preserve part of a sample for the analysis, the conveyance, storage and preparation must not to be such as to bring about any significant change in the results of the analysis.
### Tab 14. Reference methods of measurement (CD 79/869/EEC, Annex I)

<table>
<thead>
<tr>
<th>Microbiological parameters</th>
<th>Limit of detection</th>
<th>Materials recommended for containers</th>
<th>Method of analysis and inspection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliforms /100 ml</td>
<td>5 (*) 500 (**)</td>
<td>Sterilized glass</td>
<td>Culture at 37 °C on an appropriate specific solid medium (such as Tergitol lactose agar, Endo agar, 0.4 % Teepol broth) with filtration (*) or without filtration (**) and colony count. Samples must be diluted or, where appropriate, concentrated in such a way as to contain between 20 and 100 colonies. If necessary, identification by gasification.</td>
</tr>
<tr>
<td>Faecal coliforms /100 ml</td>
<td>2 (*) 200 (**)</td>
<td>Sterilised glass</td>
<td>Culture at 44 °C on an appropriate specific solid medium (such as Tergitol lactose agar, Endo agar, 0.4 % Teepol broth) with filtration (*) or without filtration (**) and colony count. Samples must be diluted or, where appropriate, concentrated in such a way as to contain between 10 and 100 colonies. If necessary, identification by gasification.</td>
</tr>
<tr>
<td>Faecal streptococci /100 ml</td>
<td>2 (*) 200 (**)</td>
<td>Sterilised glass</td>
<td>Culture at 37 °C on an appropriate solid medium (such as sodium azide) with filtration (*) or without filtration (**) and colony count. Samples must be diluted or, where appropriate, concentrated in such a way as to contain between 10 and 100 colonies.</td>
</tr>
<tr>
<td><em>Salmonella (</em>**)*</td>
<td></td>
<td>Sterilised glass</td>
<td>Inoculation into pre-enrichment medium. Enrichment and transfer into isolating gelose. Identification</td>
</tr>
</tbody>
</table>

Notes:
- (*): Method of dilution with fermentation in liquid substrates in at least three tubes with three dilutions. Sub-culturing of the positive tubes on a confirmation medium. Count according to MPN (Most Probable Number). Incubation temperature 44 ± 0.5 °C.
- (**): Method of dilution with fermentation in liquid substrates in at least three tubes with three dilutions. Sub-culturing of the positive tubes on a confirmation medium. Count according to MPN (Most Probable Number). Incubation temperature 37 ± 1 °C.
(*) for waters of Category A1, G value (CD 75/440/EEC).
(**) for waters of Categories A2 and A3, G value (CD 75/440/EEC).
(**) absence in 5000 ml (A1, G) and absence in 1000 ml (A2, G) (CD 75/440/EEC).

**Shellfish Directive (CD 79/923/EEC)**

This directive aims to protect and/or improve the quality of coastal and brackish water bodies, that can support shellfish life (bivalve and gastropod molluscs) and to contribute to the high quality of shellfish products directly edible by man.

It states that MS shall designate those coastal and brackish waters to be considered as shellfish waters and it sets the minimum quality criteria, which must be met by shellfish waters in terms of:

- physico-chemical and microbiological parameters
- mandatory limit values (I) and the guide values (G) of these parameters
- minimum sampling frequency and the reference methods of analysis of these waters.

MS shall set the standard values in accordance with the guidelines in the directive. They may set more stringent values than those laid down in the directive, but they shall not set values less stringent than I values (Imperative values) and shall endeavour to observe the G values (Guideline values).

Water shall be considered as complying with the provisions of the directive if samples, taken at the minimum frequency specified and at the same sampling point over a period of 12 months, conform to the values set by MS, as regards:

- 100 % of the samples for the parameters “organo-halogenated substances” and “metals”
- 95 % of the samples for the parameters “salinity” and “dissolved oxygen”
- 75 % of the samples for the other parameters (e.g. microbiological parameters).

Moreover, it has to be considered that: a) if sampling frequency for all parameters, except “organo-halogenated substances” and “metals”, is lower than that indicated, values and comments shall be complied for all the samples; b) if the quality of waters is appreciably higher (but the exact value is not mentioned) than that specified, the frequency of the sampling might
be reduced; c) if there is no pollution and no risk of deterioration in the quality of the waters, sampling may be not necessary.

The sampling point, the distance from this point to the nearest point where pollutants are discharged and the depth at which samples are to be taken is decided by MS on the basis of local environmental conditions. The directive sets out reference methods of analysis. Nevertheless, laboratories can employ other methods provided that they ensure that the results obtained are equivalent or comparable to those specified.

*Criteria for microbiological analysis at source*

The directive stipulates a requirement of 300 faecal coliforms per 100 ml of shellfish flesh and interstitial fluid (table 15). However, since in 1979 a directive on the protection of consumers of shellfish products had not yet been adopted, it additionally states that this value has to be observed in waters from which shellfish are taken for direct human consumption.

**Tab 15. Microbiological standard for shellfish waters (CD 79/923/EEC)**

<table>
<thead>
<tr>
<th>Microbiological parameters</th>
<th>G</th>
<th>I</th>
<th>Minimum sampling frequency</th>
<th>Method of analysis and inspection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal coliforms /100 ml</td>
<td>300</td>
<td></td>
<td>Every 3 months</td>
<td>Method of dilution with fermentation on liquid substrates in at least three tubes in three dilutions. Sub-culturing of the positive tubes on confirmation medium. Count according to MPN (most probable number). Incubation temperature 44 ± 0.5 °C</td>
</tr>
</tbody>
</table>

Nowadays, a distinct Directive (*Shellfish Hygiene Directive, CD 91/492/EEC*) is entirely aimed to protect consumers of shellfish. It lays down health conditions for the production and placing on the market of live bivalve molluscs that are intended for immediate human consumption or for further processing before consumption.

Microbiological standards are set in terms of concentrations of coliform bacteria and *Salmonella.*
Live bivalve molluscs intended for immediate human consumption must comply with the following microbiological requirements:

- contain less than 300 faecal coliforms or less than 230 *E. coli* per 100 g of mollusc flesh and interstitial liquid based on a five-tube, three-dilution MPN-test. Any other bacteriological procedure shown to be of equivalent accuracy can be used

- not contain *Salmonella* in 25 g of mollusc flesh

- in the absence of routine virus testing procedures and the establishment of virological standards, health checks must be based on faecal bacteria counts.

The effectiveness of faecal indicator bacteria and their numerical limits as well as other parameters laid down must be kept under constant review and, where scientific evidence proves the need to do so, be revised. Examinations for checking compliance with these requirements must be carried out in accordance with methods scientifically recognised.

Microbiological standards are set out also for production areas defined as: sea, estuarine, or lagoon areas containing natural deposits of bivalve molluscs or sites used for cultivation of bivalve molluscs from which live bivalve molluscs are taken. According to the directive, different types of bivalve production areas can be classified based on the treatment required for live bivalve molluscs in order to be appropriate for human consumption.

A) Areas from which live bivalve molluscs can be collected for direct human consumption. Molluscs taken from these areas must meet the requirements previously set out for immediate human consumption.

B) Areas from which live bivalve molluscs can be collected but only placed on the market for human consumption after treatment in a purification centre. Live bivalve molluscs from these areas must not exceed the limits of a five-tube, three-dilution MPN-test of 6000 faecal coliforms per 100 g of flesh or 4600 *E. coli* per 100 g of flesh in 90% of samples. After purification or relaying, all the requirements set out for immediate human consumption must be met.

C) Areas from which live bivalve molluscs can be collected but placed on the market only after relaying over a long period (at least two months), irrespective of whether this is combined with purification, or after intensive purification for a period to be fixed in order to meet the
requirements under A). Live bivalve molluscs from these areas must not exceed the limits of a five-tube, three-dilution MPN-test of 60000 faecal coliforms per 100 g of flesh.

**Information exchange Directive (CD 77/795/EEC)**

This directive establishes a common procedure for the exchange of information on the quality of *surface fresh water* in the Community.

The Directive locates “sampling or measuring stations” for each MS and lays down surface fresh water parameters whose data have to be forwarded to the Commission by means of a central agency designated in each MS. The information, covering a calendar year, shall be forwarded to the Commission at least every 12 months and the Commission shall draw up annually a consolidated report based on this information.

The information concerning parameters (CD 77/795/EEC, Annex II) shall be:

- the results of the measurements carried out by the sampling or measuring stations
- a description of the sampling, sample preservation and measuring methods used and the frequency of sampling.

Parameter values shall be presented according to the modes of expression and with the significant figures set out in the following table. Both the list of the stations and of the parameters can be amended.

**Tab 16. Microbiological parameters in respect of which information is to be exchanged (modes of expression and significant figures for the parametric data) (CD 77/795/EEC, Annex II)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Modes of expression</th>
<th>Significant figures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal coliforms</td>
<td>/100 ml</td>
<td>xxxxxxx</td>
</tr>
<tr>
<td>Total coliforms *</td>
<td>/100 ml</td>
<td>xxxxxxx</td>
</tr>
<tr>
<td>Faecal streptococci *</td>
<td>/100 ml</td>
<td>xxxxxxx</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>/1 litre</td>
<td>x</td>
</tr>
</tbody>
</table>

*Data relating to this parameter shall be exchanged when it is measured*
### Tab 17. Reference methods of measurement (CD 77/795/EEC, Annex III)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mode of expression</th>
<th>Reference methods and measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal coliforms</td>
<td>/100 ml</td>
<td>Culture at 44 °C on an appropriate specific solid medium (such as Tergitol lactose agar, Endo agar, 0.4 % Teepol broth) with or without filtration and colony count. Samples must be diluted or, where appropriate, concentrated in such a way as to contain between 10 and 100 colonies. If necessary, identification by gasification. Method of dilution with fermentation liquid substrates in at least three tubes in three dilutions. Sub-culturing of the positive tubes on a confirmation medium. Count according to MPN (most probable number). Incubation temperature: 44 ± 0.5 °C.</td>
</tr>
<tr>
<td>Total coliforms</td>
<td>/100 ml</td>
<td>Culture at 37 °C on an appropriate specific solid medium (such as Tergitol lactose agar, Endo agar, 0.4 % Teepol broth) with filtration or without filtration and colony count. Samples must be diluted or, where appropriate, concentrated in such a way as to contain between 20 and 100 colonies. If necessary, identification by gasification. Method of dilution with fermentation liquid substrates in at least three tubes in three dilutions. Sub-culturing of the positive tubes on a confirmation medium. Count according to MPN (most probable number). Incubation temperature: 37 ± 1 °C.</td>
</tr>
<tr>
<td>Faecal streptococci</td>
<td>/100 ml</td>
<td>Culture at 37 °C on an appropriate solid medium (such as sodium azide) with or without filtration and colony count. Method of dilution in sodium azide broth (Litsky). Count according to MPN (most probable number)</td>
</tr>
<tr>
<td>Salmonella</td>
<td>/1 L</td>
<td>Concentration by filtration (on membrane or appropriate filter) Inoculation into pre-enrichment medium. Enrichment and transfer into isolating gel ease-identification</td>
</tr>
<tr>
<td>Biological quality</td>
<td>Pending Community wide harmonisation, MS will use their respective methods</td>
<td></td>
</tr>
</tbody>
</table>
Directives NOT containing microbiological standards


Council Directive 76/464/EEC concerns the pollution caused by certain dangerous substances discharged into the aquatic environment (inland surface waters, territorial waters, inland coastal waters and ground water). In 1980 protection of groundwater was taken out and regulated under the Directive 80/68/EEC. Two classes of compounds are introduced, named “list I” (black list) and “list II”. MS are required to eliminate water pollution caused by list I substances and to reduce pollution caused by list II substances. Discharges of list I substances in water require prior authorisation by the competent authority of the MS. The permits must contain emission limit values restricting the maximum concentration and quantity of the substances and shall be reviewed regularly and renewed when necessary. Limit values applicable to substances within list I are laid down on the basis of their toxicity, persistence and bioaccumulation. In 1982 the Commission communicated to the Council (Official Journal C 176, 14.6.1982) a list that includes 129 “candidate list I substances”; three more substances were subsequently added. However, until now, only 18 individual substances have been regulated. Their emission limit values and quality objectives have been set in 5 “daughter” directives (CD 82/176/EEC, CD 83/513/EEC, CD 84/156/EEC, CD 84/491/EEC and CD 86/280/EEC, as emended by CD 88/347/EEC and CD 90/415/EEC). Regulation of other “candidate list I substances” has been suspended due to the preparation of an integrated permitting system for industrial installations. In 1996, the IPPC Directive (CD 96/61/EC) on integrated pollution prevention and control included the emission limits for these 18 substances as minimum requirements for large installations.

List II contains “list I substances” (114) not regulated on Community level and substances that have a deleterious effect on aquatic environment. MS must establish pollution reduction programmes including deadlines for implementation, prior authorisation and compliance with emission standards based on water quality objectives.
Water Framework Directive set out the transitional provisions for CD 76/464/EEC:

- article 6 (list I substances) will be repealed with the entry in force of the WFD
- "list of priority substances" will replace the "candidate list I substances"
- "rest" of the Directive CD 76/464/EEC will be still in place until 2013

**Groundwater Directive (CD 80/68/EEC)**

The purpose of this directive is to **prevent** the pollution of groundwater caused by certain dangerous substances (families and groups of substances in lists I or II of the directive) and, as far as it is possible, to check or **eliminate** the consequences of pollution which has already occurred.

Discharges of domestic effluents from isolated dwellings, discharges containing very small quantities of substances listed in CD 80/68/EEC and discharges of matter containing radioactive substances are excluded.

**List I** contains substances belonging to organohalogen compounds, organophosphorus compounds, organotin compounds, substances which possess carcinogenic mutagenic or teratogenic properties in or via the aquatic environment, mercury and its compounds, cadmium and its compounds, mineral oils, hydrocarbons, cyanides with the exception of those which are considered inappropriate to list I on the basis of a low risk of toxicity, persistence and bioaccumulation.

**List II** contains some metalloids and metals and their compounds, biocides and their derivatives, substances with a deleterious effect on the taste and/or odour of groundwater, toxic or persistent organic compounds of silicon, inorganic compounds of phosphorus and elemental phosphorus, fluorides, ammonia and nitrites.

MS shall **prohibit** discharge of substances in **list I** and **limit** the introduction of substances in **list II** into groundwater. To comply with this obligation all indirect discharges of list I substances and direct or indirect discharges of list II substances are subject to prior authorisation. Such authorisation is granted, for a limited period and subject to regular review, after an investigation into the receiving environment and lays down the conditions that have to be met for discharges.
However, should prior investigation reveal that the groundwater into which the discharge of substances in list I is envisaged is permanently unsuitable for other uses, especially domestic or agricultural, the MS may authorise the discharge of these substances provided that their presence does not hinder exploitation of ground resources. MS may take more stringent measures than those provided for under this directive.

**Freshwater fish Directive (CD 78/659/EEC)**

This directive concerns the protection and/or improvement of the quality of running or standing fresh waters which support or which, if pollution were reduced or eliminated, would become capable of supporting certain fish species. Waters in natural or artificial fish ponds used for intensive fish farming are excluded. MS are required to designate the fresh waters which are to be considered suitable for fish breeding. These waters are subdivided into salmonid waters and cyprinid waters:

- **Salmonid waters** (salmon and trout): generally fast flowing stretches of river that have high oxygen content and low level of nutrients
- **Cyprinid waters** (coarse fish, carp, tench, barbel, rudd, roach): slower flowing waters, which often flow through lowlands

MS are required to set the values which they will apply to such waters in accordance with the guidelines stated in the Directives. They may set more stringent requirements.

Two types of standards are set for salmonid and cyprinid waters:

- **Imperative (I) values**: standards that must be met if the stretch is to pass the Directive (for the stretch to be “compliant”). Values have been set for dissolved oxygen, pH, non-ionised ammonia, total ammonium, total residual chlorine, zinc and (for thermal discharges) temperature
- **Guideline (G) values**: quality standards that should be achieved where possible. Values have been set for chemical parameters, such as copper, biochemical oxygen demand and suspended solids

In exceptional circumstances, such as storms or droughts, derogations (waivers) may be granted for certain substances and the required standards may be exceeded.
The directive lays down the minimum quality criteria to be met by such waters, such as: physical, chemical parameters, binding limit values and indicative values for these parameters, minimum frequency of sampling and reference methods of analysis.

**Urban Wastewater Treatment Directive (CD 91/271/EEC)**

This directive sets minimum standards for the collection, treatment and discharge of urban wastewater, with the aim to reduce pollution of raw water by domestic sewage, industrial wastewater and rainwater run-off. It also prohibits the practice of dumping sewage sludge at sea. Primary, secondary and tertiary treatments are defined.

MS should provide sewage collection systems (to all towns with a population of 2000 or more) and treatment systems of urban wastewater meeting criteria laid down in the directive. MS have to draw up lists of sensitive and less sensitive areas, which receive the treated waters in order to define the treatment of urban water according to the sensitivity of the receiving body. Discharges from urban wastewater treatment plants shall satisfy relevant requirements in terms of BOD₅, COD and total suspended solids (facultative) and in terms of nitrogen and phosphorus, with great attention to sensitive areas (areas subjected to eutrophication). Values for maximum admissible concentration or for minimum percentage of reduction, with respect to the load of the influent, shall be applied. Under the directive, wastewater is normally subjected to a secondary treatment, while a tertiary one is required for discharge to particularly sensitive areas (areas subjected to eutrophication and surface waters intended for abstraction for drinking water).

**Nitrates Directive (CD 91/676/EEC)**

The purpose of this directive is the identification of vulnerable zones (affected by pollution by nitrates) and the establishment of programs to prevent pollution in order to safeguard drinking water supplies and prevent ecological damage. It covers fresh water, particularly that used for drinking water abstraction and water bodies that are or may become eutrophic. It is particularly stressed the impact of nitrates pollution on eutrophication of both inland and coastal waters.
MS are required, under this directive, to identify zones affected by pollution by nitrates (vulnerable zones) and to establish codes of good agricultural practice to be implemented by farmers on a voluntary basis. Moreover, MS must establish and implement action programmes for vulnerable zones, including measures to limit the spreading on land of any fertiliser containing nitrogen and to set limits for the spreading of livestock effluent. The directive authorises MS to take additional measures or to reinforce the action programmes.

References

Mission of the JRC

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.