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SCIENTIFIC OPINION

Scientific Opinion on the minimum hygiene criteria to be applied to clean seawater and on the public health risks and hygiene criteria for bottled seawater intended for domestic use[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2,4]

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ABSTRACT

Microbiological hazards have been associated with seawater. Poor quality sea water may consequently have a severe impact on public health. Coastal sources used for abstraction of seawater cannot be classified as a pristine source. The use of water safety plans, combining sanitary surveys with microbiological criteria and appropriate water treatment, is proposed in order to ensure adequate hygiene conditions and to control hazards. The comprehensiveness of the sanitary survey, the stringency of microbiological criteria, and the need for treatment depend on the relative exposures associated to the different uses of seawater. For uses with low exposure to microbiological hazards, a basic sanitary survey and microbiological criteria based on the Directive 2006/7/EC are considered appropriate. For uses with a higher exposure, a more comprehensive sanitary survey, mandatory water treatment, and microbiological criteria based on Council Directive 98/83/EC with an additional criterion for *Vibrio* spp. are considered appropriate. For uses with highest exposure, a more comprehensive sanitary survey, mandatory water treatment, and microbiological criteria based on Council Directive 98/83/EC with an additional criterion for turbidity and *Vibrio* spp. are considered appropriate. Both inorganic and organic chemicals can be found in seawater in concentrations that are usually low. Therefore the use of seawater on fresh or processed fishery products or for re-vitalisation of live molluscs is unlikely to raise a health concern. A potential health concern may occur from the domestic use of bottled seawater where human exposure might be expected to be higher than for the other uses of seawater. Therefore, the concentration of chemicals in bottled seawater should comply with the standards laid down in Council Directive 98/83/EC on the quality of water intended for human consumption. It is recommended to use ultraviolet (UV) or other physical methods as the

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preferred disinfection process to prevent the formation of hazardous disinfection by-products such as bromate and trihalomethanes.

KEY WORDS

Bottled seawater, clean seawater, hygiene criteria, public health risk

SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards (BIOHAZ) and the Panel on Contaminants in the Food Chain (CONTAM) were asked deliver a scientific opinion on the minimum hygiene criteria to be applied to clean seawater and on the public health risks and hygiene criteria for bottled seawater intended for domestic use.

European food legislation establishes the conditions for the use of clean seawater in land-based fishery establishments. Currently, the use of clean seawater is allowed for use in on-shore establishments, auctions and fish markets, for the handling and washing of fishery products, the production of ice for chilling fishery products and for rapid cooling of crustaceans and molluscs after their cooking. Nonetheless, there are no harmonised rules in the European legislation with regard to the sanitary criteria that clean seawater should respect.

Based on incidents of food and waterborne infection, the properties and the distribution of the agents, microbiological hazards (including viruses, bacteria and parasites) have been associated with seawater. Poor quality sea water may consequently have a severe impact on public health through contamination which may occur during food processes. The hazards are associated either with bacteria, which are part of the natural marine biota (in particular *Vibrio* spp.), or pathogenic microbes derived from animal or human faecal contamination, which is most often of terrestrial origin. Nonetheless, there is currently not sufficient data on microbiological hazards to estimate the public health risks associated with the uses in on-land establishments for handling and washing fishery products, for the production of ice used for chilling, for rapid cooling of crustaceans and molluscs after cooking, and for bottled seawater. In the absence of data to propose risk-based criteria, hazard-based criteria are proposed instead. These should provide the same level of health protection as achieved by other food business operators through the use of potable water.

It is underlined that coastal sources, used for abstraction of seawater in land-based establishments, cannot be guaranteed to be free from pathogens from the marine biota or from faecal contamination, and cannot be classified as a pristine source.

Sanitary surveys provide information to optimize the site of abstraction in order to control sources of faecal pollution and chemical contamination. Additional safeguards will be needed to reduce contamination from endogenous marine flora (including pathogenic *Vibrio* spp. and *C. botulinum*). Since these hazards are associated with temperature and salinity (*Vibrio* spp.) as well as sediments (*C. botulinum)*, abstracting seawater with high salinity (especially in waters of temperatures below 20 ºC), and free from particulate material will improve safety of seawater prior to treatment. The comprehensiveness of the sanitary survey, the stringency of microbiological criteria and the need for treatment will depend on the relative exposures associated to the different uses of clean seawater. When seawater is used for purposes that do not involve a direct contact with food (physical cleaning operations of utilities, surfaces, floors, equipment in facilities such as fish markets, auctions, fishery ports) or do not convey a contamination risk with prepared fishery products (e.g. handling and washing whole fishery products), it is considered that the exposure will be low. For this use, a basic sanitary survey and microbiological criteria based on the Directive 2006/7/EC are considered appropriate. Higher exposure to microbiological hazards will occur where seawater will be in contact with prepared, processed and/or ready-to-eat fishery products. For these uses, a more comprehensive sanitary survey, mandatory water treatment and microbiological criteria based on Council Directive 98/83/EC and an additional criterion for *Vibrio* spp. are considered appropriate. Highest exposure to microbiological hazards occurs where seawater is used for revitalisation of live bivalve molluscs, as a component of salad dressings or other ready-to-eat products. For these uses, a more comprehensive sanitary survey, mandatory water treatment and microbiological criteria based on Council Directive 98/83/EC for water offered for sale in bottles and an additional criterion for turbidity and *Vibrio* spp. are considered appropriate.

For verification of treatments, detection methods for *E. coli* and enterococci are defined in the international standards (ISO 9308-3 or ISO 9308-1 for *E. coli* and ISO 7899-1 or ISO 7899-2 for enterococci). Reference methods for the detection of *Vibrio* in seafood (ISO/TS 21872-1:2007 or ISO/TS 21872-2:2007) should be applied to seawater with appropriate modification.

Both inorganic and organic chemicals can be found in seawater in concentrations that are usually low. Therefore the use of seawater on fresh or processed fishery products or for re-vitalisation of live molluscs is unlikely to raise a health concern. A potential health concern may occur from the domestic use of bottled seawater where human exposure might be expected to be higher than for the other uses of seawater, indicating that more rigid criteria are needed for bottled seawater.

In line with the requirements for food business operators to use water of potable water quality laid down in Regulation 853/2004 it is concluded that the same approach should be applied for bottled seawater which will be placed on the market. Therefore the concentration of chemicals in bottled seawater should comply with the standards for chemicals (parameter values) as laid down in Council Directive 98/83/EC on the quality of water intended for human consumption.

The Directive addresses a drinking water consumption of 2 l per day by a 60 kg adult. No data on the consumption of bottled seawater are available, but it can be assumed that this will be much less. Therefore applying the criteria laid down in Council Directive 98/83/EC will provide a high level of protection for consumers using bottled seawater.

For nearly all chemicals the reported levels in seawater are below the respective parameter values laid down in the Council Directive 98/83/EC, indicating that there is no health concern. For boron, however, a mean value of 3.6 mg/l (range 0.7 – 4.9 mg/l) has been reported, which is well above its parameter value of 1 mg/l in Council Directive 98/83/EC, and also above the World Health Organization (WHO) guideline value of 2.4 mg/l. Therefore operators should measure boron levels in seawater and make an assessment of whether these levels might pose a risk for human health, given the consumption of bottled seawater, and should consider whether treatment with a selective boron ion exchange resin is needed to bring the boron concentration below its parameter value of 1 mg/l.

No data were identified on the occurrence of acrylamide, epichlorohydrin and vinyl chloride in seawater. Since these compounds are particularly used in drinking water treatment and transport, it can be expected that the concentration in seawater will be low. It is therefore recommended that operators determine the levels of these chemicals in seawater to investigate whether continuous monitoring is needed.

Bromate and trihalomethanes are disinfection by-products related to the use of, respectively, ozonation or chlorination. Because of its high bromide content these by-products may be more easily formed in seawater than in fresh water. When ultraviolet (UV) or other physical methods such as filtration are used as disinfection method these compounds will not be formed. It is therefore recommended to use these methods as the preferred disinfection process.

The presence of toxic algae in source water, particularly in coastal water may pose a potential health risk for the consumer. However, due to their size algae can effectively be removed by sand or (micro) filtration. It is, however, possible that a certain number of toxic algae cells or toxins, if the cells are disrupted, can settle on whole or freshly prepared fishery products. It should be noted that in that case levels of marine toxins will be much lower than those reached by bio-accumulation of toxins in bivalve molluscs or fish.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 852/2004 of the European Parliament and of the Council on the hygiene of foodstuffs[5] defines in Article 2 (h) 'clean seawater' as "*natural, artificial or purified seawater or brackish water that does not contain micro-organisms, harmful substances or toxic marine plankton in quantities capable of directly or indirectly affecting the health quality of food*".

Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin[6] authorises in its Annex III, Section VIII point 3(c) the use of clean seawater for the handling and washing of fishery products, the production of ice for chilling fishery products and for rapid cooling of crustaceans and molluscs after their cooking. The use of clean seawater for washing or purifying live bivalve molluscs in purification and dispatch centres is also allowed by the same Regulation (Annex III, Section VII, Chapter IV, A1 and B2).

In addition the same Regulation prohibits in Annex III, Section VII, Chapter VIII, point 2, that live bivalve molluscs be re-immersed in, or sprayed with, water after they have been packaged for retail sale and left the dispatch centre.

In the food law legislation there are no harmonised rules regarding the sanitary criteria that clean seawater should respect, leaving the responsibility to the Member States to fix those criteria.

However, harmonised criteria on the quality required for shellfish waters are established in Directive 2006/113/EC of the European Parliament and of the Council of 12 December 2006.[7] Shellfish waters are those coastal and brackish waters designated by the Member States as needing protection or improvement in order to support shellfish (bivalve and gastropod molluscs) life and growth and thus to contribute to the high quality of shellfish products directly edible by man.

Some Member States were recently asked by certain food business operators to allow on the EU market bottled clean seawater for domestic use (e.g. cooking or "re-vitalisation" of live bivalve molluscs at home). Based on the information received, it seems that the seawater is submitted to "purification and filtration" before being placed on the market.

In view of the above, the Commission needs to set harmonised hygiene criteria applicable to clean seawater for use in on-land establishments for the handling and washing of fishery products, the production of ice for chilling fishery products and for rapid cooling of crustaceans and molluscs after their cooking as foreseen in the hygiene Regulation.

In addition, the Commission is in need of a scientific opinion concerning the risks to public health represented by the use of bottled clean seawater for domestic use and the hygiene criteria to be applied.

[5] OJ L 139, 30.4.2004, p. 1
[6] OJ L 139, 30.4.2004, p. 55
[7] O.J.L 376, 27.12.2006 p.14

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29(1) of Regulation No.178/2002, EFSA is requested:

- to identify the minimum hygiene criteria to be applied to clean seawater for use in on-land establishments for the handling and washing of fishery products, the production of ice for chilling fishery products and for rapid cooling of crustaceans and molluscs after their cooking as foreseen in Annex III, Section VIII point 3(c) to Regulation (EC) No 853/2004,

- to assess the public health risks of bottled clean seawater and to identify the hygiene criteria (bacteriological, viral and chemical) to be respected for domestic uses such as cooking or the "re-vitalisation" at home of live bivalve molluscs including the treatment that should be applied to this seawater before being placed on the market,

- to identify the more appropriate detection methods to be used in routine analyses to verify the compliance with the above hygiene criteria.

ASSESSMENT

1. Introduction

European food legislation establishes the conditions for the use of clean seawater in land-based fishery establishments. Currently the use of clean seawater is allowed in on-shore establishments, auctions and fish markets, for the handling and washing of fishery products, the production of ice for chilling fishery products and for rapid cooling of crustaceans and molluscs after their cooking. The use of clean seawater for washing or purifying live bivalve molluscs in purification and dispatch centres is also allowed[8] but it is outside the scope of this mandate.

Although Regulation (EC) No 853/2004[9] originally limited the use of seawater specifically to whole products and, on board ships, to gutted and headed fishery products, transitional provisions were arranged that permitted the use of clean seawater, until 31 December 2009, for all other uses (manufacture of ice and handling of fishery products in on-shore establishments and auctions, for cooling of cooked shellfish and molluscs). Article 11 of Commission Regulation (EC) No 2076/2005,[10] provides that clean seawater may also be used in land-based establishments until 31 December 2009. This transitional arrangement has now become permanent within Regulation (EC) No 1020/2008, which states that the use of clean seawater for the handling and washing of fishery products does not represent a risk for public health as long as control procedures based on HACCP principles are developed and put in place by food business operators. Nonetheless, there are no harmonised rules in the European legislation with regard to the sanitary criteria that clean seawater should respect.

The Codex Alimentarius Commission's Code of practice for fish and fishery products (CAC/RCP 52-2003[11]) recommends the use of clean seawater for several operations in the fish industry, such as washing fish prior to filleting or cutting; washing fillets after filleting, skinning or trimming to remove any signs of blood, scales or viscera; washing of filleting equipment and utensils to minimize building up of slime, blood and offal; and after splitting. Seawater may be used also for washing of whole cephalopods and cephalopod products, and for cooling cooked crustaceans. The definition of clean seawater used in this document is similar to the one used in EU legislation. Codex Alimentarius defines 'clean sea water' as seawater which meets the same microbiological standards as potable water and is free from objectionable substances.

No criteria exist in the European legislation with respect to the use of seawater directly by consumers. Domestic uses of seawater such as cooking shellfish or "re-vitalisation" of live bivalve molluscs are new purposes that should be evaluated. The World Health Organization (WHO) has recently published the fourth edition of the guidelines for drinking-water quality, where drinking-water safety, including minimum procedures and specific guideline values and how these are intended to be used, are considered (WHO, 2011). However no indication is included in relation to the use of seawater by the consumer or the food industry.

The framework for identifying adequate hygiene conditions for seawater has been to endorse the use of water safety plans that, when implemented by the food business operator, provide the basis for ensuring that seawater for the intended uses contain a number of pathogens and concentrations of chemicals that represent a minimal exposure to consumers. Options to control hazards in clean

[8] These provisions are set out in Regulation (EC) No 852/2004, Part A of Annex I and in Chapter VII of Annex II, and in Part II of Chapter I and Chapters III and IV of Section VIII of Annex III, in particular for handling fishery products on board vessels. OJ L 139, 30.4.2004, p. 1–54.

[9] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L 139, 30.4.2004, p.55-205.

[10] Commission Regulation (EC) No 2076/2005 of 5 December 2005 laying down transitional arrangements for the implementation of Regulations (EC) No 853/2004, (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004. OJ L 338, 22.12.2005, p. 83–88.

[11] www.codexalimentarius.net/download/standards/10273/CXP_052e.pdf

seawater include a combination of sanitary surveys, with microbiological criteria and appropriate water treatment based on the multiple-barrier principle. The stringency of microbiological criteria depends on the relative exposures associated to the different uses of clean seawater; less stringent criteria to be applied to clean seawater intended for handling, washing and chilling of whole fishery products and more stringent criteria to be applied to clean seawater intended for handling, washing and chilling of prepared and/or processed fishery products, and for rapid cooling of crustaceans and molluscs after their cooking, and for bottled seawater for domestic use. It is proposed that the less stringent criteria are based, e.g. on Directive 2006/7/EC. More stringent criteria should provide equivalent public health protection to potable water standards, e.g. based on Council Directive 98/83/EC.[12] Because of the characteristics of the marine environment, to ensure meeting potable water standards it is considered appropriate to subject the seawater to treatment. Minimum hygiene criteria were requested only for on-land establishments, according to terms of reference. The use of seawater on board of vessels is not covered in this document, neither is the use of bottled seawater outside domestic environments.

2. Description of uses of seawater

Clean seawater can be used in the food industry for the following purposes:

- Cleaning of facilities and equipment. Seawater can be used in establishments such as fish markets, on-shore establishments and auctions. Seawater is used to remove gross organic material and wastes from gutting equipment and utensils by hosing and to minimize build-up of slime, blood and debris.

- Manufacture of ice for cooling and storage of fishery products, either fresh or processed. It is used on ships, on-shore establishments and auctions, fish markets, etc., to cool down whole fish. Seawater ice has a slightly lower melting point than freshwater ice with the advantage of cooling fish at a slightly faster rate and to a lower temperature. It melts faster than freshwater ice and consequently has to be replenished more often. Another advantage of seawater ice is its ability to be easily manufactured on shore or at sea where freshwater ice may not be readily available. Refrigerated seawater is also used to chill large quantities of fish. It is generally used with mechanical refrigeration units that cool seawater to below 0 °C. In some cases, brine or water of similar salinity to seawater is used. Seawater mixed with ice is known as chilled seawater and allows products to be cooled rapidly while reducing mechanical damage during packaging and handling.

- Washing of whole fishery products is a normal practice in auction facilities, but also on ships before unloading. Whole fish are washed in seawater by hosing during sizing and grading, to remove loose scale, foreign waste and reduce bacterial load prior to gutting.

- Washing after operations such as gutting, beheading, skinning or trimming is done to remove blood, viscera, and scales. Hosing filleting equipment and utensils removes blood and offal and minimizes the build-up of slime. All these operations can be done using clean seawater as well as potable freshwater.

- Washing and cooling of crustaceans and molluscs after cooking. Cooking is followed by rapid cooling which can be done in different ways, such as using refrigeration chambers, or less commonly, using refrigerated seawater or brine. Cooling must be implemented until a temperature approaching that of melting ice is reached.

- Water supply for fish and crustacean tanks.

[12] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.98, p. 32-54.

Bottled seawater is a new product which is being marketed by a few food business operators in the EU. It can be used for culinary purposes at domestic level (e.g. cooking or "re-vitalisation" of live bivalve molluscs at home) and is advertised for use in the following domestic food preparation activities:

- Cooking of fish, molluscs, crustaceans, and pasta.
- Adding as a constituent of dough for bread baking, pizzas or savoury pastries.
- As a component of salad dressing (seawater/oil and seawater/vinegar).

According to the producers, bottled sea water may undergo treatment for example by filtration (5 µm) and UV irradiation or microfiltration.

3. Hazard identification and characterisation

The approach for hazard identification has been to include those food- or waterborne biological hazards that may be present in seawater due to either being naturally occurring pathogens (autochthonous microbiota), or due to faecal contamination from human or animal sources (sewage/surface runoff/rivers and streams and/or direct contamination by human activity such as bathing). Also chemicals, either from natural or anthropogenic origin that may be present in seawater, and toxic algae that might pose a health concern, are included in the hazard identification.

3.1. Microbiological hazards

3.1.1. Norovirus

Noroviruses (NoV) belong to the family *Caliciviridae*, that is divided into genera. NoV and *Sapovirus* are the two out of five genera of the family *Caliciviridae* that contain viruses that cause infections in humans. NoV have also been detected in pigs, cattle, mice, cats, dogs, and sheep, and sapoviruses in pigs. The other genera of the family *Caliciviridae* are *Lagovirus*, *Vesivirus*, and *Nebovirus* encompassing viruses infecting rabbits, and brown hares (lagoviruses), sea lions, swine, cats, dogs, fish, seals, other marine animals, cattle and primates (vesiviruses), and cattle (*Nebovirus*) (EFSA Panel on Biological Hazards (BIOHAZ), 2011).

3.1.1.1. Survival/growth in ice, fishery products and seawater

Noroviruses are unable to grow outside a permissive host (in this instance humans) but their transmission can be through consumption of filter-feeding shellfish which demonstrates their ability to survive in seawater (Carter, 2005; EFSA Panel on Biological Hazards, 2011). Infectivity in shellfish may not be reduced after one month storage at 4ºC, after freezing or in ice (Butot et al., 2008; Carter, 2005).

3.1.1.2. Data linking presence in seawater to food-borne illness

Calicivirus (including norovirus) causes approximately 90 % of epidemic non-bacterial outbreaks of gastroenteritis around the world and is responsible for many foodborne outbreaks of gastroenteritis. The virus is transmitted by food or water contaminated with human faeces and by person-to-person contact. Outbreaks of norovirus disease often occur in closed or semi-closed communities, such as long-term care facilities, hospitals, prisons, dormitories, and cruise ships where once the virus has been introduced, the infection spreads very rapidly. Many norovirus outbreaks have been traced to food that was handled by one infected person (EFSA and ECDC, 2011).

In the EU summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks 2009 information on the food vehicle was provided for all but one of the 43 verified outbreaks caused by calicivirus (including norovirus). In contrast to previous years, where crustaceans, shellfish, molluscs and products thereof, and buffet meals were the most frequently associated food vehicles, fruit, berries, juices and other products thereof were the major food vehicle in 2009, implicated in 22 outbreaks. Other relevant food vehicles were vegetables and juices and products

thereof (six outbreaks, 254 cases) and mixed or buffet meals (two outbreaks, 204 cases). The settings that were most often reported were restaurants, households, schools and kindergartens and canteen or workplace catering and residential institutions (EFSA and ECDC, 2011).

Probably the best known presentation of NoV is that of large outbreaks of vomiting and diarrhoea, that led to the initial description of "winter vomiting disease" (Mounts et al., 2000). The majority of NoV gastroenteritis cases results from direct person-to-person transmission. However, NoV related outbreaks have been shown to be food- or waterborne, caused by for example, contaminated shellfish (Doyle et al., 2004; Kingsley et al., 2002; Le Guyader et al., 2003), raspberries (Ponka et al., 1999) or drinking water (Carrique-Mas et al., 2003; Kukkula et al., 1999; Parshionikar et al., 2003).

A challenging question is how much disease caused by noroviruses can be attributed to foodborne spread. It is clear that the major mode of transmission for noroviruses remains person-to-person (de Wit et al., 2001b; Fretz et al., 2005; Karsten et al., 2009; Pajan-Lehpaner and Petrak, 2009). Due to the high rate of secondary transmissions, small initial foodborne events may rapidly present like person-to-person outbreaks, if the initial introduction event was not recognized. In The Netherlands, approximately 12-15 % of community cases of NoV gastroenteritis were attributed to foodborne transmission, based on analysis of questionnaire data, and this has been used in later burden of disease estimates. In the EU summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks 2009 outbreaks were stratified into possible and verified foodborne outbreaks, where epidemiological evidence for a food source or detection of the pathogen in food is considered as evidence. Only 5 % of NoV outbreaks have been labelled as verified, which reflects the difficulties in detecting NoV in food items.

3.1.1.3. Adverse health effects and incidence

In humans, NoV and sapoviruses cause gastroenteritis, while in other animals these viruses can cause a range of different clinical syndromes, including oral lesions, systemic disease with hemorrhagic syndromes, upper respiratory tract infections. So far, the NoV and sapoviruses are the only caliciviruses known to cause disease in humans, with the exception of anecdotal zoonotic infection with vesiviruses.

Few studies have looked at the incidence and health impact of NoV infection at the community level. The most extensive data are from the UK (Tam et al., 2012; Tompkins et al., 1999; Wheeler et al., 1999) and the Netherlands (de Wit et al., 2001b), where a randomised sample of the community participated in cohort studies of infectious intestinal disease (IID). The incidence of community-acquired IID was calculated as 274 per 1000 person years in the UK (Tam et al., 2012) and 283 per 1000 person years in The Netherlands (de Wit et al., 2001a; Tompkins et al., 1999). Viruses were the most frequently identified causes of community acquired gastroenteritis, with NoV detected in 11 % of cases in The Netherlands and 7 % in the UK. NoV infection is common in all age groups but the incidence is highest in young children (<5 yrs). In recent years, the incidence of norovirus outbreaks has increased with the emergence of new variants, in particular genogroup II (Lopman et al., 2004).

3.1.1.4. Conclusion on importance in seawater in comparison to other sources and possible inclusion in microbiological criteria

There is no microbiological evidence proving a link between Norovirus presence in seawater and human disease through direct ingestion of contaminated seawater. A separate criterion for Norovirus is not considered necessary. Indicator organisms are likely to provide a better indication of faecal contamination of seawater.

3.1.2. Hepatitis A virus

The etiological agent of hepatitis A is the hepatitis A virus (HAV) which belongs to genus *Hepatovirus* within family *Picornaviridae*, and as such it consists of a non-enveloped icosaedral capsid of around 30 nm in diameter containing a positive ssRNA genomic molecule of 7.5 Kb (Fauquet et al., 2005). A single serotype of HAV has been so far reported.

3.1.2.1. Survival/growth in ice, fishery products and seawater

HAV is a highly stable virus, able to persist for extended times in the environment (Abad et al., 1994a; Abad et al., 1994b; Sobsey et al., 1988) and its transmission by contaminated foods and drinking water has been demonstrated (Bosch et al., 1991; Dentinger et al., 2001; Pinto et al., 2009; Reid and Robinson, 1987; Rosemblum et al., 1990; Sanchez et al., 2002), although most cases seem to occur through person-to-person transmission. Foods of primary importance are those susceptible to be contaminated at the pre-harvest stage such as bivalve molluscs, particularly oysters, clams and mussels, salad crops, such as lettuce, green onions and other green leafy vegetables, and soft fruit, such as raspberries and strawberries. All these types of food have been implicated in foodborne HAV outbreaks (CDC, 1997; Halliday et al., 1991; Pinto et al., 2009; Shieh et al., 2007; Wheeler et al., 2005). Epidemiological data therefore indicate that infectivity is retained within shellfish. The virus can also retain infectivity following freezing or in ice (Butot et al., 2008; Carter, 2005).

3.1.2.2. Data linking presence in seawater to food-borne illness

The first documented shellfish-borne outbreak of "infectious hepatitis" occurred in Sweden in 1955, when 629 cases were associated with raw oyster consumption (Roos, 1956). However, the most significant outbreak of HAV infection occurred in Shanghai, China, in 1988, in which almost 300,000 cases were caused by consumption of clams harvested from a sewage-polluted area (Halliday et al., 1991). This is the largest virus-associated outbreak of food poisoning ever reported. Depurated shellfish have been associated with outbreaks of norovirus, hepatitis A gastroenteritis, and other viral diseases (Conaty et al., 2000). The virus has also been associated with the consumption of contaminated fresh-cut vegetables and fruit. The EU summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks 2009 reported one verified food-borne outbreak due to Hepatitis A virus (EFSA and ECDC, 2011).

3.1.2.3. Adverse health effects and incidence

The prevalence of hepatitis A in different geographical areas of the world is closely related to their socioeconomic development (Gust, 1992; Hollinger and Emerson, 2007; Previsani et al., 2004). The endemicity is low in industrialised regions and high in other parts of the world. The epidemiological pattern has important implications on the average age of exposure and hence, as above stated, on the severity of the clinical disease. Since hepatitis A infection induces a life-long immunity (Hollinger and Emerson, 2007), severe infections among adults are rare in highly endemic regions where most children are infected early in life. In contrast, in low endemic areas the disease occurs mostly in adulthood, mainly as a consequence of travelling to endemic regions, having risky sexual practices or consuming contaminated water or food; and hence the likelihood of developing severe symptomatic or fatal illness is high.

The Hepatitis A virus is distinguished from other viral agents by its prolonged (two to six weeks) incubation period and its ability to spread beyond the stomach and intestines into the liver. It often induces jaundice, or yellowing of the skin, and in rare cases leads to chronic liver dysfunction.

3.1.2.4. Conclusion on importance in seawater in comparison to other sources and possible inclusion in microbiological criteria

There is no microbiological evidence proving a link between Hepatitis A virus presence in seawater and human disease through direct ingestion of contaminated seawater. A separate criterion for Hepatitis A virus is not considered necessary. Indicator organisms are likely to provide a better indication of faecal contamination of seawater.

3.1.3. *Salmonella*

Salmonella is a facultatively anaerobic, Gram-negative bacterium that can cause illness in humans and animals. *Salmonella* are excreted in the faeces of animals (including birds) and humans that are infected with, or asymptomatically excreting, the organism. Strains of *Salmonella* Typhi/Paratyphi

cause enteric fever, a serious systemic illness. Non-typhoidal *Salmonella* cause gastroenteritis in humans.

3.1.3.1. Survival/growth in ice, fishery products and seawater

Studies using seawater inoculated with high concentrations of *Salmonella* have shown that while the T90 value is in the order of one day, the organism can survive for extended periods in this matrix: the period varies with the recovery protocol but ranges from approximately one year to twenty years (Dhiaf et al., 2010; Morinigo et al., 1990; Sugumar and Mariappan, 2003). *Salmonella* Senftenberg has been reported to persist in the marine environment for more than five years (Martinez-Urtaza and Liebana, 2005). However, the survival of *Salmonella* Typhi in similar experiments has been reported much shorter with less than 0.1 % survival after three days (Nabbut and Kurayiyyah, 1972). Growth of *Salmonella* may occur if nutrients are present in the seawater. *Salmonella* does survive in frozen foods (FSA, 2003; Ripabelli, et al., 2004), however the extend of survival may be affected by the food matrix.

Salmonella has been reported to be present in up to 12.2 % of raw fish and 2.6 % of ready-to-eat fishery products (Heinitz et al., 2000). A study of imported fish in Japan showed approximately 30-40 *Salmonella* cells/100g (Asai et al., 2008) and multiplication will therefore increase the risk of infection. *Salmonella* is a mesophilic organism and the growth rate of this organism is markedly reduced at temperatures above 15°C while the growth of most strains is prevented at temperatures above 7°C (ICMSF, 1996). However, most studies on minimum growth temperature were investigated in products other than seafood. *S.* Heidelberg has been reported to have a generation time of 28h and 31h in the English sole and sterile crab respectively at 8˚C (ICMSF, 1996). Growth of *Salmonella* occurred in cooked crab inoculated with *Salmonella* and stored at 11˚C both in air and under modified atmosphere containing 50 % CO_2 (Ingham et al., 1990). No growth was seen in either atmosphere at 7 °C. *Salmonella* have the ability to proliferate at pH values ranging from 3.8 to 9.5 with optimum being 7.0-7.5 (ICMSF, 1996). Growth of *Salmonella* is generally inhibited at 3-4 % NaCl, but salt tolerance increases with increasing temperature in the range 10-30˚C (D'Aoust and Maurer, 2007) and minimum water activity for growth is 0.94 (ICMSF, 1996).

The EU summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks 2009 (EFSA and ECDC, 2011) included data on samples tested for *Salmonella*. Twelve MSs and Norway reported investigations of *Salmonella* in fish and fishery products with 25 samples or more. An overall percentage of 0.3 % of the tested samples was positive for *Salmonella*, which was at the same level as in 2008. Three MSs (Germany, Italy and Spain) reported positive samples. For Germany and Spain this was at a very low level but Italy reported one specific investigation with 73 samples of unspecified fishery products, where six samples were positive (8.2 %). Concerning molluscan shellfish and live bivalve molluscs, a total of 4,819 samples (from eight MSs) were tested in investigations with 25 samples or more, and 1.1 % of these were positive. Spain found the highest level of contamination with 3.9 % of live bivalve molluscs being positive (N=358). Norway tested 92 samples of raw molluscan shellfish with no positive. Not all reports on molluscan shellfish include information on whether the sampled items were cooked, raw and/or ready-to-eat. Tests on crustaceans were reported by seven MSs (with 25 samples or more). Only one out of a total of 1,437 samples was positive. This was one out of 686 single samples at retail reported by Germany

3.1.3.2. Data linking presence in seawater to food-borne illness

The proportion of salmonellosis outbreaks attributed to fishery products is generally much lower than that for many other groups of foodstuffs (in the range of 1 to 2.5 % of reported outbreaks) and thus fishery products are not considered a significant vehicle for this illness (FAO, 2010).

A dose-response relationship has been developed that shows that the probability of infection changes from 1:400 for the ingestion of one cell to 1:2 for the ingestion of 10,000 cells (FAO/WHO, 2002). The model relates to foodborne infection and may not be directly applicable for waterborne infections: there will be no matrix-protective effect for the bacteria with direct ingestion in water.

Contaminated drinking water is widely accepted as an important risk factor in the transmission of typhoid (WHO, 2006). There are also reports of non-typhi *Salmonella* spp. being linked to illness, either through contaminated drinking water itself or ice made from this (Taylor et al., 2000; WHO, 2006). There is some evidence linking typhoid with gross sewage pollution of both fresh and marine recreational waters (Parker, 1990; PHLS, 1959). A WHO report on health risks from recreational waters presented no evidence linking non-typhoidal salmonellosis with saline recreational waters (Pond, 2005).

A German study showed that *Salmonella* were present in 12.3 % of samples taken from North Sea sites outside designated bathing waters but not in any of samples taken from such designated bathing waters (Tobias and Heinemeyer, 1994). In other studies from Spain, Morocco and Mexico, the proportion of seawater samples positive for *Salmonella* spp. varied from 2.3 to 4.1 % (Martinez-Urtaza et al., 2004; Setti et al., 2009; Simental and Martinez-Urtaza, 2008). Efstratiou et al. (2009) showed that levels of either total coliforms or faecal coliforms adequately predicted the likelihood of the presence of *Salmonella* in seawater samples, although enterococci did not. However, others have argued that *E. coli* is not a suitable indicator for *Salmonella* in the environment (Winfield and Groisman, 2003). This may well be the case in those situations where certain strains of *Salmonella* persist in marine ecosystems for extended periods.

3.1.3.3. Adverse health effects and incidence

Transmission of *Salmonella* to humans is predominantly via water or food contaminated with faecal material, or cross-contaminated from other products containing the organism, or contaminated by infected food-handlers. Strains of *Salmonella* Typhi/Paratyphi cause enteric fever, a serious systemic illness. Incubation period ranges from 7 to 28 days. Symptoms include malaise, headache, fever, cough, nausea, vomiting, constipation, abdominal pain, chills, rose spots, bloody stools. Strains of non-typhoidal *Salmonella* may cause gastroenteritis in humans. Incubation period ranges from 8 to 72 hours. Symptoms include abdominal pain, diarrhoea, chills, fever, nausea, vomiting and malaise. Systemic infection such as septicaemia may occur especially in susceptible patients such as the very young, very old and immune-compromised.

3.1.3.4. Conclusion on importance in seawater in comparison to other sources and possible inclusion in microbiological criteria

There is little evidence to support seawater as a significant source of salmonellosis. An exception may be the acquisition of typhoid in endemic areas from seawater grossly contaminated with sewage. In most circumstances any criteria for faecal indicator bacteria should indicate the likelihood of *Salmonella* contamination. A separate criterion for *Salmonella* is not considered necessary.

3.1.4. *Vibrio cholerae, parahaemolyticus* and *vulnificus*

Vibrios are natural inhabitants of marine systems with a worldwide distribution. The different species of this genus show specific ecological preferences and consequently have different habitat distributions.

3.1.4.1. Survival/growth in ice, fishery products and seawater

Vibrios are native organisms of marine environments throughout the world and form part of the indigenous microflora of the environment at the time of seafood capture or harvest. *V. parahaemolyticus*, *Vibrio cholerae*, and *Vibrio vulnificus* are resident of estuarine environments and their boundaries of distribution are strongly associated with the gradient of salinity. *V. cholerae* occupies freshwater and brackish habitats, whereas *V. vulnificus* is present in environments with intermediate levels of salinity and *V. parahaemolyticus* shows preference by more saline environments and may be present in off-shore waters. Due to the affinity for areas of moderate salinity, the distribution of these three species is primarily confined to estuaries and coastal areas with salinity values below 30 ppt. In these areas, vibrios show a complex life cycle governed by seasonal variations of seawater temperature (Joseph et al., 1982). Pathogenic vibrios show a preference for warm waters

and their numbers in the environment remain low when water temperatures drop below 16 °C with highest abundance at temperatures above 20 °C (West, 1989). Coastal areas are also the most important areas of fishing and shellfish production and the presence of pathogenic vibrios in seafood can result in major public health concerns.

Pathogenic vibrios may be found on the skin, chitinous shell, gills as well as the intestinal tracts of fish or shellfish (ICMSF, 1998). Subsequent improper handling and the absence of a bactericidal step (e.g. cooking) may raise the level of bacteria in the final product and present a health risk to consumers. Molluscan bivalves are filter feeders and accumulate microorganisms from their surrounding waters, which may also contain vibrios. They are usually grown and harvested in near-shore and estuarine waters and are therefore likely to harbour high concentrations of naturally occurring organisms, including pathogenic vibrios. As they can be eaten raw or after a very mild heat treatment, they constitute a significant health risk to the consumers, if contaminated (Gram and Huss, 2000).

V. vulnificus is usually associated with seafood from estuarine or coastal marine environments with warm water temperatures and moderate salinity, such as the southern coastal US States. Although *V. vulnificus* is most often associated with filter-feeding shellfish, the organism can be present in fish and other marine products. *V. vulnificus* is present in waters, sediments, plankton, molluscs, crustaceans and finfish, for example within estuaries of the Gulf Coast of the USA (FAO/WHO, 2005). A recent study in India highlighted the presence of *V. vulnificus* in the tropical waters of the southwest coast of India (Parvathi et al., 2004).

V. cholerae will grow rapidly in temperature-abused foods and will also survive for extended periods in chilled and frozen foods, but it does not survive desiccation for more than 48 hours. The cells are not heat-resistant and are readily destroyed by cooking and pasteurisation. *V. parahaemolyticus* differs from *V. cholerae* in that it is an obligate halophile and will not grow unless a salt concentration of at least 0.5 % is present. Like *V. cholerae, V. parahaemolyticus* will grow rapidly in temperature-abused foods and survives chilled and frozen storage, but not drying or mild heat processes. *V. vulnificus* is a halophile requiring at least 0.5 % salt to grow and will tolerate levels of up to 5 %. It multiplies in live oysters, but not at temperatures of less than 13˚C. Like other species it resists low temperatures for some time, but is destroyed by cooking and is not resistant to desiccation.

3.1.4.2. Data linking presence in seawater to food-borne illness

Vibrio is recognized as a primary cause of bacterial gastroenteritis associated with seafood consumption in many areas of the world, including Asia and the U.S. The number of infections related to these pathogens has increased since 1990. Vibrios are concentrated in the gut of filter-feeding molluscan shellfish, such as oysters, clams and mussels, where they can multiply. The majority of food-borne illness is caused by *V. parahaemolyticus*, *Vibrio cholerae*, or *Vibrio vulnificus*. *V. parahaemolyticus* and *V. cholerae* are mainly isolated from gastroenteritis cases associated with consumption of seafood (both species) or contaminated water (*V. cholerae*). In contrast, *V. vulnificus* is primarily reported from extra intestinal infections (septicaemia, wounds, etc.) and may be associated with consumption of seafood in some specific geographical areas.

Pathogenic vibrios have been detected in a broad range of seafood products in which they can survive different processes. Foods associated with illnesses due to consumption of *V. parahaemolyticus* includes crayfish, lobster, shrimp, fried mackerel, mussel, tuna, seafood salad, raw oysters, clams, steamed/boiled crabmeat, scallops, squid, sea urchin, mysids (shrimp-like crustaceans), and sardines (FAO/WHO, 2011). These products include both raw and partially treated (heat treatment, high pressure) and thoroughly treated seafood products exposed to cross-contamination through contaminated utensils, hands, etc. Improper refrigeration of seafood contaminated with *Vibrio* may allow the proliferation of bacterial cells, with a corresponding increased risk of infection.

Endogenous marine species of *V. cholerae* can be isolated from unprocessed fish during cholera outbreaks, although it is more likely for contamination of food to occur either using water containing

these bacteria (e.g. where sewage contamination has occurred) during processing or following manipulation by handlers carrying the pathogen (Lawley et al., 2008). Contaminated water used to make ice can lead to the contamination of beverages. In the industrial world, *V. cholerae* infections are usually associated with the consumption of seafood. Shellfish can become contaminated from environmental sources and most non-O1/O139 cholera infections are associated with the consumption of raw oysters. Other foods implicated in *V. cholerae* infections are fruit and vegetables, grains, meat and legumes (Lawley et al., 2008).

Although thorough cooking destroys these organisms, oysters are often eaten raw and, at least in the US, are the most common food associated with *V. parahaemolyticus* infection (Hlady, 1997). At present, strains producing *tdh* and *trh* are considered pathogenic to humans (FAO/WHO, 2011). *V. parahaemolyticus* is an important source of foodborne disease, especially in Japan and other Asian countries. *V. parahaemolyticus* is part of the normal microflora of coastal and estuarine waters in almost all temperate regions and may be present in comparatively high numbers when the water temperature is at its highest during the summer. In Europe *V. parahaemolyticus* infections are rarely reported. However, a growing number of cases and outbreaks have been reported over the last year in Spain, Israel and Baltic region in relation to the warming of coastal waters (Baker-Austin et al., 2010).

Humans are the main reservoir for *V. cholerae* and cholera is usually associated with poor hygiene and polluted water supplies, but may also be foodborne. Contamination of fruit, vegetables and other foods usually occur via an infected food handler, or by the use of polluted water in food preparation. Strains causing classic epidemic cholera generally belong to one of two serogroups, O1 or O139, although non-O1/O139 strains can cause a less severe form of diarrhoeal disease. These isolates are typically related to consumption of contaminated shellfish, especially raw oysters. Non-O1/O139 *V. cholerae* strains are common in certain estuarine waters and may be present on shellfish.

V. vulnificus is an occasional cause of serious infections, which may sometimes be foodborne. However, wound infection following contact with the marine environment is more likely. *V. vulnificus* is now the leading cause of death in the USA related to consumption of seafood and is almost always associated with raw oysters from the Gulf of Mexico, which are thought to have a very high contamination rate. Only about 90 cases of *V. vulnificus* infection are reported each year in the USA and major outbreaks have not been recorded. Infections are rare and generally limited to individuals with pre-existing chronic illnesses, including those with liver disorders, or immunocompromised. In this cases of particular risk, *V. vulnificus* can invade through the intestinal barrier into the bloodstream causing primary septicaemia. As a result, *V. vulnificus* infections have the highest case/fatality rate (approx. 50 %) among foodborne pathogens (FAO/WHO, 2005). Elsewhere, sporadic cases have been identified in Europe, Korea and Taiwan.

The dependence of *Vibrio* infections on the ingestion of large numbers of cells reduces the risk of infection associated with the contamination of seafood through seawater. The number of *Vibrio* cells on a product contaminated through seawater is expected to be low and, the risk of *Vibrio* illness from consumption of primary product may be considered primarily as low.

However, the high growth rate of *Vibrio* species can result in a high risk of infection. Cases of illness caused by *V. parahaemolyticus* have occurred when seafood has been cross-contaminated by raw fish after cooking and growth of the bacterium has occurred following subsequently temperature abuse. Implicated seafood in outbreaks includes clams, oysters, scallops, shrimp and crab. *V. parahaemolyticus* has one of the shortest generation times of any bacterium (<10 min) with an optimum growth temperature of approximately 37 °C. Inappropriate temperature control of seafood can lead to a rapid increase in the number of viable *Vibrio* cells on contaminated products and there will be a high risk of causing disease. One of the largest outbreak of *Vibrio* illness in Europe was related to the use of contaminated seawater to chill boiled crabs (Martinez-Urtaza et al., 2005). The processor premise was located in an important seaport with heavy international traffic of cargo, cruise and fishing vessels, which may have introduced the pandemic *V. parahaemolyticus* through ballast

water. The circumstances involved in the processing of the crabs suggested that the potential sources of contamination were associated with the harbour water employed in post-processing management.

Information regarding the effectiveness of common water disinfection treatments against pathogenic Vibrios remains scarce. Whereas some treatment systems have proved to be efficient in reducing the load of bacteria in water, evaluation of oxidising disinfectants have shown that chlorine and ozone are ineffective in preventing biofilm formation and in removing mature biofilms formed by *Vibrio* species in seawater and seafood premises (Shikongo-Nambabi et al.). Furthermore, attachment to particulate matter, aggregation, encapsulation of the pathogen, ingestion by protozoa, and water turbidity may affect chlorine efficacy (CDC, 2008). Chlorination of water used in blower tanks has been found inefficient for the elimination of *V. cholerae* from oysters during depuration (Motes, 1982). Rugose variants of *V. cholerae* O1 showed a high resistance to chlorination whilst retaining virulence (Morris et al., 1996).

3.1.4.3. Adverse health effects and incidence

Ingestion of a large number of viable cells is needed for pathogenic *Vibrio* spp. to survive the acidic environment of the stomach and establish an infection. Food matrix factors such as fat levels, acidity, salt content, and other characteristics can have a significant influence on the competence of *Vibrio* to cause disease. Other factors related to the host such as the general health status or physical stress can play an important role in the individual response to infections. The immune status, especially of those individuals who are immunocompromised can influence occurrence and/or severity of *Vibrio* diseases.

Most of the pathogenic strains of *V. cholerae* and *V. parahaemolyticus* recovered from human infections possess specific traits. Virulent populations of *V. cholerae* and *V. parahaemolyticus* may be discriminated from non-virulent strains based on the presence of specific genetic markers or/and by their ability to produce major virulence factors. *V. parahaemolyticus* strains recovered from human gastroenteritis cases typically carry the thermostable direct haemolysin (*tdh*) gene, the *tdh*-related haemolysin (*trh*) gene, or both. These virulence markers occur infrequently in strains isolated from environmental sources and foods. Strains bearing *tdh* or *trh* genes represent less than 3 % of all *V. parahaemolyticus* strains isolated from the environment (Depaola et al., 1990). However, the relative abundance of pathogenic strains may be substantially higher in some areas and during certain times of the year (Rodriguez-Castro et al., 2010). *V. cholerae* infections are most often associated with strains belonging to O1 and O139 serotypes which generally possess the *ctx* gene and produce cholera toxin (CT) and are responsible for classic epidemic cholera. Non-O1/O139 *V. cholerae* strains can cause a less severe form of diarrhoeal disease. Epidemic cholera is confined mainly to developing countries with warm climates. The concentration of free-living choleragenic *V. cholerae* in natural aquatic environment is low, but *V. cholerae* is known to attach and multiply on planktonic organisms. While the virulence of *V. vulnificus* has been studied extensively, there is no one virulence marker that is definitively associated with human illness as *tdh* is with *V. parahaemolyticus* (Martinez-Urtaza et al., 2010).

A quantitative evaluation of the dose-response relationship between the levels of *V. parahaemolyticus* ingested and the frequency and severity of illness was conducted in FDA Risk Assessment (FDA, 2005). The dose-response relationship for *V. parahaemolyticus* estimated from human clinical feeding trial studies and epidemiological surveillance data showed a predicted probability of illness of approximately 0.5 for a dose of approximately 100 million cfu, using the curve with the highest weight. This means that for every 100 servings at that dose level, approximately 50 individuals will become ill. At exposure levels of approximately 1,000 cfu, the probability of illness is relatively low (<0.001). The probability of illness approaches 1.0 (i.e., 100 % certainty of illness) at exposure levels around $1x10^9$ cfu.

For O1/O139 cholera, symptoms can occur between 5 h and 6 days after infection. Dose-response curves show that a high dose of choleragenic *V. cholerae* O1 (10^6) is normally needed to cause illness when choleragenic *V. cholerae* O1 are consumed in food (FAO, 2003).

3.1.4.4. Conclusion on importance in seawater in comparison to other sources and possible inclusion in microbiological criteria

Vibrios are naturally occurring organisms in marine environments and hence their presence in seawater does not correlate with indicators of faecal contamination. Despite their potential presence in coastal water, the risk of contamination of processed fishery products should be considered low where seawater is exclusively used in cleaning of premises and washing whole unprepared fish products. In these cases of low exposure, a specific criterion for *Vibrio* would not be considered applicable. However, the use of untreated seawater should be avoided in post-processing stages to enable the reduction of potential risks of *Vibrio* contamination, primarily in ready-to-eat products. In this sense, only potable water is recommended by the Codex Guidelines for *Vibrio* in Seafood (CAC/GL 73-2010) to be used after cooking and blanching seafood products and in post-harvest stages. In those cases in which the use of seawater is applied in operations with a higher exposure (prepared and/or processed fishery products, rapid cooling of crustaceans and molluscs after their cooking and bottled seawater) an additional microbiological criterion for total *Vibrio* spp. would be applied to ensure the effectiveness of treatment systems in removing potential pathogenic vibrios from seawater.

3.1.5. *Listeria*

Listeria monocytogenes is a bacterium causing severe systemic infection in humans (Farber and Peterkin, 1991). The disease is predominantly transmitted by consumption of contaminated foods, and is one of the major causes of death from a preventable foodborne illness.

3.1.5.1. Survival/growth in ice, fishery products and seawater

The bacterium will grow between below 0 °C and 44 °C, at 10 % NaCl, over the pH range 5 to 9. D values have been reported as: 60 ºC range 2 to 17 minutes; 70 ºC 6-14 seconds; and 71.7 ºC 0.9 to 5 seconds (Jay et al., 2005). Survival in seawater has been reported for a few days, but will be dependent on the temperature as well as amount of UV exposure (Bremer et al., 1998; Hansen et al., 2006; Hsu et al., 2005). Survival in frozen products is well documented, including in fishery products (Ritz et al., 2008).

In the EU summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks 2009 (EFSA and ECDC, 2011) 14 MSs reported data on findings of *L. monocytogenes* in ready-to-eat fish products. The products tested were mainly smoked fish. The presence of *L. monocytogenes* in fish products was detected in 12 out of 14 qualitative investigations. In 2009, a total of 2066 samples were tested qualitatively and 7.0 % were found positive for *L. monocytogenes*, compared to 9.8 % in 2008. Relatively high proportions of *L. monocytogenes* positive samples (qualitative examinations) were reported at retail by Slovenia with 35.0 % of 40 samples of smoked fish positive, and by Finland with 28.1 % positive of 64 samples of gravad fish products packaged in a vacuum or modified atmosphere. Five of 12 investigations reported levels of *L. monocytogenes* above 100 cfu/g. Overall, 0.6 % of 1965 samples tested quantitatively were found to exceed the limit of 100 cfu/g, compared to 0.5 % in 2008. The proportion of samples containing the bacteria above the limit of 100 cfu/g ranged from 0.7 % to 2.5 % in samples of smoked fish from Slovenia.

3.1.5.2. Data linking presence in seawater to food-borne illness

L. monocytogenes is widespread in the environment and commonly occurs in surface waters (Colburn et al., 1990; Wilkes et al., 2011) which will consequently contaminate estuarine and coastal waters (Beleneva, 2011; Bou-m'handi et al., 2007; Colburn et al., 1990; El-Shenawy and El-Shenawy, 2006; El Marrakchi et al., 2005; Hansen et al., 2006; Rodas-Suarez et al., 2006; Rorvik et al., 1995). Consumption of contaminated processed fish and shellfish has been associated with transmission of infection (Brett et al., 1998; Ericsson et al., 1997; Facinelli et al., 1989; Farber et al., 2000; Lyytikainen et al., 2006; Misrachi, 1991; Mitchell, 1991; Riedo et al., 1994; Tham et al., 2000). The fish and shellfish implicated in disease transmission were processed, ready-to-eat, able to support the growth of this bacterium and likely to have been contaminated at the point of processing, although the presence of the bacterium in raw product can not be excluded as a source of contamination in food

processing environments. The faeces of wild birds is also a potential source of *Listeria* contamination in marine environments (Fenlon, 1985).

3.1.5.3. Adverse health effects and incidence

L. monocytogenes causes severe systemic illness especially to those of 60 years of age, the pregnant woman and unborn infant and immunocompromised: there has been an increase in the numbers of reports within the EU largely confined to those over 60 (Denny and McLauchlin, 2008). The EU summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks 2009 (EFSA and ECDC, 2011) estimated over 1,380 cases reported within the EU, with a rate of 0.3 cases per 100,000 population in 2008. The proportion of cases attributable to the consumption of marine products in the EU is not known.

3.1.5.4. Conclusion on importance in seawater in comparison to other sources and possible inclusion in microbiological criteria

L. monocytogenes is widespread in terrestrial environments and the major hazard is from contamination of processed ready-to-eat foods in production environments. The importance of contamination of seawater is probably minor and consequently this bacterium is not considered further in this document for inclusion in microbiological criteria.

3.1.6. *Clostridium botulinum*

Botulism is an intoxication caused by the ingestion of a potent neurotoxin which occurs in foods resulting from the germination and growth of *Clostridium botulinum*. The bacterium is a Gram-positive, spore-forming rod whose spores are widely distributed in the environment. It can be present in processed food by contamination of raw materials or by post-processing contamination. The spores are heat-resistant and can survive in foods that are insufficiently processed. Seven serotypes (A-G) have been described (based on the neurotoxin antigenicity) but only types A, B, E and F are regularly associated with human foodborne botulism cases.

3.1.6.1. Survival/growth in ice, fishery products and seawater

The spores of *C. botulinum* are naturally distributed in the environment including water (fresh and seawater) and consequently are likely to contaminate fishery products. Spores have a high resistance to environmental stresses (heat, starvation, freezing, osmotic stress, drying) and their survival in water is extreme. *C. botulinum* type E is the most common type found in fresh water and marine environments. Types A and B are generally found on land, but may also be occasionally found in water. *C. botulinum* type E is part of the natural flora of aquatic environments and occurs in sediments of lakes, ponds and sea where anoxic conditions and carrion occur. *C. botulinum* type E can possibly multiply or at least, survive, in anoxic sediments. The presence of *C. botulinum* in water is usually as the result of low water levels (in ponds or shallow waters), disturbances of mud or sediments, or any condition that causes high fish mortality. There is also a significant correlation between off-shore bottom oxygen content, depth, and bioturbation activity with overall prevalence and spore counts of *C. botulinum* type E in aquatic sediments. *C. botulinum* spores in the sea are mainly confined to temperate and arctic aquatic environments. Several studies show that the predominant type in water is *C. botulinum* type E which, for example, was found in 81 % of sea and 61 % of freshwater samples, taken from aquatic environments of the Baltic Sea and Finnish mainland (Hielm et al., 1998b).

3.1.6.2. Data linking presence in seawater to food-borne illness

No reports are known linking botulism to water consumption although in theory waterborne botulism, from the ingestion of the pre-formed toxin, could occur. Water is not an appropriate environment for germination and toxin production

C. botulinum type E has been isolated from fish gills, skin and intestines and of trout or salmon which can act as a transient carrier of spores (Alahuikku et al., 1977; Burns and Williams, 1975; Hielm et al., 1998a; Hielm et al., 2002; Huss and Eskildsen, 1974; Pullela et al., 1998). Botulism outbreaks

associated with fish are usually linked to non-proteolytic *Clostridium botulinum* type E and have been reported in the northern and temperate regions. Type E has been frequently implicated in fishborne intoxications due to consumption of industrially processed raw smoked salmon and trout (Bach and Mueller-Prasuhn, 1971; Baumgart, 1970; Dressler, 2005; Hauschild and Gauvreau, 1985). Other *C. botulinum* types such as type A, B and C have also been occasionally isolated from fish (Austin and Dodds, 1996; Baker et al., 1990), but not linked to fishborne botulism.

3.1.6.3. Adverse health effects and incidence

Botulism intoxication onset usually starts 18 to 36 hours after ingestion of food containing the neurotoxin. Symptoms are due to the toxin binding the receptors on nerve endings and preventing the release of acetylcholine at the neuromuscular junction, which causes the progressive paralytic symptoms typical for botulism, such as weakness, double vision, vertigo, and progressive difficulty in speaking and swallowing. Difficulty in breathing, weakness of other muscles, abdominal distension, and constipation may also be progressive symptoms.

An average of 450 outbreaks of foodborne botulism are reported annually worldwide (Hatheway, 1995). Thirty-four per cent of the outbreaks were due to type A, 52 % to type B, and 12 % to type E. Countries with relatively high occurrences of foodborne botulism are China, Iran, the United States, Germany, France, Poland and Italy (Hauschild and Gauvreau, 1985; Hauschild, 1992). It is remarkable that a close association between the frequency and type of botulism outbreaks and the occurrence of *C. botulinum* in the environment has been shown (Dodds et al., 1989; Hauschild, 1989). *C. botulinum* type E is linked to fishborne outbreaks and it shows a high prevalence in cold or temperate regions of the northern hemisphere (Hauschild, 1992).

3.1.6.4. Conclusion on importance in seawater in comparison to other sources and possible inclusion in microbiological criteria

The spores of *C. botulinum* are widely distributed and they show a geographical distribution. They can be present in seawaters coming from contaminated environments or where sediments are disturbed. Inclusion in the microbiological criteria seems unsubstantiated.

3.1.7. *E. coli* causing intestinal illness

Many warm-blooded animals, including humans carry *Escherichia coli* in their intestines as they are part of the normal gut flora. *E. coli* is a member of the coliform group, part of the family *Enterobacteriaceae*, and is facultative anaerobic, Gram-negative, non-spore-forming rod. Most *E. coli* are harmless, yet there are several types of *E. coli* strains that may cause gastrointestinal illness in humans. These types can be divided into several pathogroups: Enteropathogenic (EPEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC), Enterohaemorrhagic (EHEC), Enteroaggregative (EAEC) and Diffusely-adherent (DAEC) *E. coli*.

3.1.7.1. Survival/growth in ice, fishery products and seawater

The presence of pathogenic *E. coli* in seawater is associated with faecal contamination from the animal or human reservoirs. Their survival capacity in aquatic habitats is very variable as some of the cells can enter a viable but non-culturable status. Survival depends on factors such as amount of solar radiation, water temperature, presence of organic mater and adequate nutrients, presence of bacteriophages, autochthonous microbiota, protozoa predation, osmotic stress, etc. Adapted cells also show more survival capacity (Garcialara et al., 1993), and there is a genetic response of cells to the environmental challenges mentioned above (salinity, starvation, pH etc.), which is mostly mediated by the rpoS regulon (Rozen and Belkin, 2001). It should be noted that differences in resistance to environmental conditions between subgroups of *E. coli* have been reported. They occupy various ecological niches, and can be broadly characterized as either commensals or pathogens (van Elsas et al., 2011). It should be noted that faecal concentrations of the typical non-pathogenic *E. coli*, used to indicate recent faecal contamination, will always be greater than those of the pathogenic strains.

Several studies highlight that ETEC should be taken into consideration in endemic areas when assessing the role of marine environments as a source of enteric infection.

3.1.7.2. Data linking presence in seawater to food-borne illness

E. coli is a commonly found in the gastrointestinal tract of humans and animals and has traditionally been used as an indicator of faecal contamination when found in the environment, water or food. As an indicator, the presence of *E. coli* in a food implies that enteric pathogens may also be present. However, it has become evident that the presence or absence of faecal pathogens cannot be directly correlated with detection or apparent absence of indicator *E. coli* (Pierson et al., 2007). At best, the presence of *E. coli* in food or water is an indication of recent faecal contamination, poor hygiene and careless handling. Although *E. coli* is a reasonably good indicator for vegetative bacterial pathogens commonly found in fresh or sea water systems, it has proven to be a poor indicator of presence of pathogenic viruses and protozoa.

Waterborne (fresh water) outbreaks of diseases caused by pathogenic strains of *E. coli* have been described, but no information is available linking seawater to *E. coli* outbreaks.

3.1.7.3. Adverse health effects and incidence

Depending on the type of *E. coli*, foodborne infection can present several different symptoms including abdominal cramps, watery or bloody diarrhoea, fever, nausea and vomiting, while enterohaemorrhagic *E. coli* (EHEC) can also cause haemorrhagic colitis and the haemolytic uremic syndrome (HUS).

3.1.7.4. Conclusion on importance in seawater in comparison to other sources and possible inclusion in microbiological criteria

Enteropathogenic *E. coli* are a minor fraction of the species *E. coli* and their presence in seawater results from faecal contamination. This group of bacteria is present in seawater in very low numbers and has historically been assumed to die off rapidly in the seawater environment. Microbiological procedures for detection and enumeration of specific pathogroups of *E. coli* are impractical for use in routine monitoring of water and cannot be considered appropriate for inclusion in microbiological criteria.

3.1.8. Thermophilic *Campylobacter* spp.

Thermophilic *Campylobacter* spp. are excreted by all warm-blooded animals, and are widespread in the environment. Occurrence in sea water may result from direct or indirect faecal contamination from humans, mammals or birds, or indirectly through wastewater discharges, surface run-off or contaminated surface waters. Decimal reduction times in sea water are in the order of one to two days, but are greatly decreased by exposure to sunlight (Sinton et al., 2007).

While farm animals and other mammals are usually colonised by the major human pathogenic species *C. jejuni* and *C. coli*, different species are typically found in wild birds, including *C. lari*, a rare human pathogen (Hughes et al., 2009). Sea gulls that feed on human waste have been reported to carry *C. jejuni*, although genetic differences with strains from humans and broiler chickens have been reported (Ramos et al., 2010). A group of water/wildlife isolates of *C. jejuni* was found to belong to a separate clade by multilocus sequence typing which are uncharacteristic of human food chain-associated isolates (Hepworth et al., 2011).

3.1.8.1. Survival/growth in ice, fishery products and seawater

Campylobacter spp. are susceptible to environmental stresses and their survival is typically less than indicator organisms as demonstrated e.g. by Sinton et al. (2007) for sunlight inactivation in seawater.

3.1.8.2. Data linking presence in seawater to food-borne illness

In 2009, one of 16 verified food-borne outbreaks of campylobacteriosis was attributed to the consumption of crustaceans, shellfish, molluscs and products thereof. No outbreaks of campylobacteriosis have been linked to consumption of fish (EFSA and ECDC, 2011).

3.1.8.3. Adverse health effects and incidence

Thermophilic *Campylobacter* spp. may cause severe diarrhoeal illness in humans, which is characterised by acute enteritis and abdominal pain lasting for up to seven days or longer. Although such infections are generally self-limiting, complications can arise and may include bacteraemia, Guillain–Barré syndrome, reactive arthritis, inflammatory bowel disease, and irritable bowel syndrome (EFSA, 2011).

Thermophilic *Campylobacter* spp. are the most frequently reported cause of diarrhoeal illness in the EU with approx. 200,000 reported cases in 2009 (EFSA and ECDC, 2011). It is estimated, however, that the true incidence of campylobacteriosis is approximately nine million cases per year. Handling and consumption of contaminated poultry meat is an important transmission pathway, accounting for 20-30 % of all cases in the EU. Other established risk factors are raw milk, drinking water, and contact with animals. 50-80 % of all human cases are caused by strains that are genetically related to those in the chicken reservoir, while ruminants (cattle, sheep) also appear to be important reservoirs (EFSA, 2010). A small fraction of human strains are genetically related to those isolated from wild birds or water.

3.1.8.4. Conclusion on importance in seawater in comparison to other sources and possible inclusion in microbiological criteria

There is little evidence to support seawater as a significant source of campylobacteriosis. In most circumstances any criteria for faecal indicator bacteria should indicate the likelihood of *Campylobacter* contamination. A separate criterion for *Campylobacter* is not considered necessary.

3.1.9. *Staphylococcus aureus*

Staphylococcus is a genus of Gram-positive bacteria which are natural residents of a wide range of mammals. Staphylococci show host specificities and *S. aureus* is a common resident of the skin and naso-mucosal flora of humans.

3.1.9.1. Survival/growth in ice, fishery products and seawater

S. aureus is environmentally resistant and will survive and grow in high concentrations of salt: generally up to 15 % but growth may occur at 20 % under some conditions. Contamination and growth in some seafood have been associated with staphylococcal foodborne disease, although the presence of *S. aureus* in food results in almost all instances from food handlers and more occasionally from animals. The bacterium survives freezing, including freezing in seafood products (Sommers and Rajkowski, 2011).

3.1.9.2. Data linking presence in seawater to food-borne illness

Since *S. aureus* is associated with carriage in humans, the bacterium occurs as a result of human activities. *S. aureus* (including meticillin-resistant *S. aureus*) has been recovered from seawater as a result of human bathing activities (Plano et al., 2011).

3.1.9.3. Adverse health effects and incidence

S. aureus causes a range of skin and soft tissue infections as well as more severe systemic disease. Food poisoning is caused by the production of enterotoxins in food prior to consumption.

3.1.9.4. Conclusion on importance in seawater in comparison to other sources and possible inclusion in microbiological criteria

Since *S. aureus* in food most often results from food handlers, the importance of contamination of seawater is probably minor and consequently this bacterium is not considered further in this document for inclusion in microbiological criteria.

3.1.10. *Shigella*

Shigella is a genus within the *Enterobacteriaceae* and comprises four species (*S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*) and is most closely related to the genus *Escherichia*. *Shigella* are human adapted and produce a range of mild to severe enteric infections, as well as more severe illnesses.

3.1.10.1. Survival/growth in ice, fishery products and seawater

Shigella are excreted in large numbers in human faeces during infection and drinking of contaminated fresh water is an important route of infection, particularly in parts of the world where untreated sewage is discharged into water courses (Niyogi, 2005). Freshwater fish may become contaminated from human sewage (Onyango et al., 2009) and survival for several days can occur in seawater (Wait and Sobsey, 2001) where seawater, marine fish and filter-feeding shellfish may become contaminated (Livingstone, 1969; Ristori et al., 2007). Survival in ice is likely to occur.

3.1.10.2. Data linking presence in seawater to food-borne illness

Contamination of freshwater with *Shigella* is important in human disease transmission, therefore prevention of contamination of foods (including seafood) is important for disease control. However, person-to-person transmission also occurs (Niyogi, 2005).

3.1.10.3. Adverse health effects and incidence

Shigellosis (bacterial dysentery) ranges in severity from mild watery diarrhoea to severe illness accompanied by febrile convulsions. The severity of illness is associated with the species involved. Infection with *S. dysenteriae* is usually most severe and some cases are also associated with the haemolytic uremic syndrome. Infection with *S. flexneri* and *S. boydii* can also be severe but *S. sonnei* in an otherwise healthy person generally presents as a few loose stools and abdominal discomfort. Shigellosis is also occasionally associated with reactive arthritis (Reiter's Syndrome).

In 2009, 7,182 confirmed case were reported in the EU, with an overall rate of 1.63 cases per 100,000 population. Shigellosis continues to be most prevalent in children under five years old and travel-associated cases, predominantly to regions outside of EU/EEA, were more frequently reported than indigenous cases (ECDC, 2011). Since *Shigella* is exclusive to humans, infection is spread by the faecal-oral route, either by direct person-to-person contact, or via contaminated food or water.

3.1.10.4. Conclusion on importance in seawater in comparison to other sources and possible inclusion in microbiological criteria

Foodborne *Shigella* infection results from contamination of foods directly or indirectly with human faeces either through contamination of water or via food handlers. Contamination of seawater is probably of minor importance as a route of transmission, and indicator organisms are likely to provide a better indication of human faecal contamination. Consequently this bacterium is not considered further in this document for inclusion in microbiological criteria.

3.1.11. *Aeromonas*

Aeromonas species (aeromonads) are ubiquitous and globally occurring aquatic organisms. Environments in which they have been detected include drinking and wastewater, the ground, surfaces, and marine water bodies. The optimum growth temperature for *Aeromonas* spp. is 22 to

35 °C, with an extended temperature range of 0 to 45 °C for some strains. The optimum pH range is from 5.5 to 9.0; a wider range of 4.5 to 9.0 is tolerated. Optimum NaCl concentration is from 0 to 4 %.

There is evidence that some species are pathogenic for humans, warm-blooded and cold-blooded animals, including domestic animals and birds. Only *A. hydrophila*, *A. veroni* biovar *sobria* and *A. caviae* are commonly isolated from clinical sources worldwide.

3.1.11.1. Survival/growth in ice, fishery products and seawater

Aeromonas spp. have been detected in a variety of foods, including raw produce, raw meat, seafood, ready-to-eat meat, dairy products, and treated drinking water. They are part of the normal microbiota of healthy animals and humans and are found at high levels in sewage (USEPA, 2006). *Aeromonas* may be ingested from water and food through the environment, but most host-microbe interactions do not result in disease. The main source of *Aeromonas* infection is water (including chlorinated water) and seafood products (D'Sa and Harrison, 2010). Other food products that also may contain enteropathogenic *Aeromonas* include poultry, raw meat, raw milk or vegetables (Burke et al., 1984a; Burke et al., 1984b).

In general, *Aeromonas* spp. do not tolerate salinities above 5 % NaCl but a few isolates tolerating 6 % have been reported (Knochel, 1990). At chill temperatures, competitive growth is not expected at levels above 3 %-4 % NaCl and a few isolates may be sensitive to concentrations as low as 2 % NaCl at sub-optimal conditions such as chill temperatures (Knochel, 1990). A few strains have been reported to be unable to grow at salt levels below 0.3 % (Palumbo et al., 1985).

Detection of aeromonads in water is also influenced by temperature and residual chlorine levels which should be greater than 15 °C and less than 0.2 mg/l respectively. The tendency of the cells to form biofilms makes accurate determination of their numbers in water systems a difficult task (USEPA, 2006).

Aeromonads are inactivated by commonly used disinfectants used in water treatment and by routinely used food processing and preparation methods. *A. hydrophila* is quite sensitive to many factors such as temperature (heating), pH, NaCl, oxygen, phosphates, etc. However, some strains of *A. hydrophila* show resistance to the usual chlorine concentrations used for treatment of drinking water (0·1–0·3 mg/l) (Massa et al., 1999). High levels of chlorine (50 μg/ml) have been proved efficient to reduce the levels of *A. hydrophila* in tomatoes (Velazquez et al., 1998).

3.1.11.2. Data linking presence in seawater to food-borne illness

A. hydrophila is a widespread representative of this genus found in water, water habitants, domestic animals and foods. *A. hydrophila* has been isolated from a wide range of both animal and plant food products, including raw red meat, poultry, fin fish, seafood, dairy products, vegetables and miscellaneous foods (Palumbo, 1996).

As a common inhabitant of water sources, drinking or mineral water can be a possible source of exposure for humans. In accordance with the Safe Drinking Water Act, *A. hydrophila* is listed in the Environmental Protection Agency's (EPA) first and second Contaminant Candidate List as a "potential waterborne pathogen".

A. hydrophila is frequently found in seafood. Wang and Silva (1999) found that from 238 channel catfish fillets, 36.1 % were contaminated with this bacterium. The incidence of this pathogen contamination was higher in the summer than other seasons. Results of a study of fresh fish from commercial outlets in France, Great Britain, Greece and Portugal (Davies et al., 2001) reported that *A. hydrophila* was detected in all these countries, with an overall incidence of 40 %. Incidences of A. *hydrophila* of 19 %, 28 %, 90 % and 22 % have been reported in fish samples from UK, New Zealand, Switzerland and Taiwan, respectively (Fricker and Tompsett, 1989; Gobat and Jemmi, 1993; Hudson et al., 1992; Tsai and Chen, 1996).

Abeyta et al. (1989) found that *A. hydrophila* in shellfish growing waters ranged from three to 4600 cells/100 g in oysters and from three to 2400 cells/100 ml in water. Colburn et al. (1989) studied the microbiological quality of oysters (*Crassostrea gigas*) and water of live holding tanks at five different Seattle area retail markets. *A. hydrophila* was the most frequently isolated potential pathogen in this study with a higher incidence in oysters (78 %) compared to water (53 %).

3.1.11.3. Adverse health effects and incidence

Evaluation of aeromonads as potential pathogens of foodborne origin dates back to the 1950s, following their isolation from humans. Their presence in water sources and raw and ready-to-eat foods supports their potential to cause outbreaks of foodborne disease under some circumstances. The most implicated species in human disease are *A. hydrophila* (48 %), followed by *A. sobria* and *A. caviae* (about 25 % each) (D'Sa and Harrison, 2010).

Aeromonas species are putative human pathogens causing gastrointestinal and other infections in healthy and immunocompromised hosts. The role of these bacteria in foodborne diseases is not firmly established, but *Aeromonas* spp. have been proposed as a potential emerging foodborne pathogens (Daskalov, 2006).

3.1.11.4. Conclusion on importance in seawater in comparison to other sources and possible inclusion in microbiological criteria

With limited general evidence identifying *Aeromonas* as causative agent of foodborne illness, a specific microbiological criterion for this bacterium is not considered necessary.

3.1.12. *Plesiomonas*

Plesiomonas shigelloides is a pathogenic bacterium native to aquatic animals and environments. Its metabolism is similar to that of the genus *Vibrio* in that sugars are fermented with acid production but no gas. This organism has been isolated from cases of human diarrheal illness, and is also suspected to be the cause of other generalized human infections (D'Sa and Harrison, 2010).

P. shigelloides grows optimally at 30 to 35 °C, as low as 10 °C, and at a pH as low as 4.5 (Jay et al., 2005). Most isolates exhibit growth from 2.0 % to 3.0 % NaCl. Some strains have been found to grow in 5.0 % NaCl (Miller and Koburger, 1986). NaCl, however, is not an absolute requirement for growth (Janda and Abbott, 1999).

3.1.12.1. Survival/growth in ice, fishery products and seawater

The primary habitats of *P. shigelloides* are fresh-water ecosystems (rivers, lakes, and surface waters) and marine estuaries in tropical and temperate climates (Monteil and Harf-Monteil, 1997). In aquatic systems, *P. shigelloides* occurs as free-living cells which can contaminate fish, crabs, shrimp, mussels, and oysters (Huber et al., 2004; Oxley et al., 2002; Schubert, 1984). It has been detected in association with river fish (Jay et al., 2005).

3.1.12.2. Data linking presence in seawater to food-borne illness

Plesiomonas has been isolated from surface waters (especially in warm weather), tap water, soil, fish, shellfish and aquatic species (Abbott et al., 1991), as well as from the intestinal contents of animals (Arai et al., 1980). Oysters are the major food incriminated in outbreaks in the United States (Levin, 2008).

The first outbreak of gastroenteritis due to *P. shigelloides* occurred in Japan in 1963 (Ueda et al., 1963) and was due to contaminated cuttlefish salad involving 275 cases of diarrhoeal infection out of 870 individuals who consumed the salad. Salted mackerel resulted in an outbreak in 1966 in Japan involving 53 cases (Hori and Hayashi, 1966). Subsequent outbreaks involved waterborne diarrhoea affecting 978 out of 2141 persons in Japan (Tsukamoto et al., 1978) and various other waterborne outbreaks (CDC, 1998; Medema and Schets, 1993) and oyster consumption (Rutala et al., 1982).

Uncooked shellfish have been found to be the most important sources of foodborne illnesses from *P. shigelloides* (Holmberg et al., 1986).

3.1.12.3. Adverse health effects and incidence

P. shigelloides produces a heat-stable enterotoxin, but based on a lack of consistent in-vitro and in-vivo evidence, the species is considered to possess low pathogenicity (Abbott et al., 1991). Some researchers believe that the organism is an opportunistic pathogen, posing a significant risk in immunocompromised patients or in those with pre-existing illness (D'Sa and Harrison, 2010).

3.1.12.4. Conclusion on importance in seawater in comparison to other sources and possible inclusion in microbiological criteria

With limited evidence that *Plesiomonas* can cause food-borne illness, typical foodborne pathogen intervention methods are effective in inactivating *P. shigelloides* in foods. The lack of evidence linking contamination of food and subsequent illness in humans supports that the organism probably poses little risk and therefore is not considered further in this document for inclusion in microbiological criteria.

3.1.13. *Cryptosporidium, Giardia,* and *Toxoplasma*

Eukaryotic parasites, particularly protozoan parasites, represent hazards to humans through exposure via marine environments. The major protozoan hazards and *Cryptosporidium*, *Giardia*, and *Toxoplasma*: all of which have complex life cycles within a variety of hosts and which produce cysts (oocysts) which have varying degrees of environmental robustness and allow survival (but not growth) outside their hosts in the environment.

Cryptosporidium is a genus of protozoan parasites which comprises 19 species and more than 44 genotypes, many of which are unlikely to be infectious to humans (Jex et al., 2011): the life cycle is monoxenous (occurs within a single host) and results in oocysts excreted into the environment via the faeces of infected animals which are immediately infectious.

Giardia is a genus of flagellate protozoan parasites of vertebrates and more than 50 species, many of which are unlikely to be infectious to humans (Thompson, 2011): the life cycle is monoxenous and results in cysts excreted into the environment via the faeces of infected animals which are immediately infectious.

Toxoplasma gondii is a species of protozoan parasite (Dubey, 2011). The life cycle requires intermediate hosts (livestock, birds and wild animals) and a definitive host (cats and other felines). Oocysts are excreted from the definitive host into the environment via the faeces and require a maturation period before becoming infectious (Fayer et al., 2004).

Other parasites (including *Sarcocystis*, *Isospora*, *Cyclospora*, *Entamoeba*, and species of microsporidia) may represent similar risks but are less well understood.

3.1.13.1. Survival/growth in ice, fishery products and seawater

All parasite cysts, particularly the oocysts of *Cryptosporidium* and *Toxoplasma*, show considerable resistance to chemical disinfectants (Dawson, 2005; Laberge and Griffiths, 1996) and are extremely robust in aquatic environments. Mild heat treatments (71.7 for 15 seconds) are effective killing steps, and some limited survival may occur in ice or at freezing temperatures (Dawson, 2005; Deng and Cliver, 1999; Fayer, 1994). (Oo)cysts of *Cryptosporidium*, *Giardia* and *Toxoplasma* are relatively sensitive to UV radiation both in the environment from sunlight and as a water treatment process (Dawson, 2005; Ware et al., 2005).

3.1.13.2. Data linking presence in seawater to food-borne illness

Very large numbers of cryptosporidial oocysts can occur in the faeces of infected animals (up to 10^9/g) and inputs into marine environments can occur via freshwater (Fayer et al., 2004) from human sewage and bathers (both *C. parvum* and *C. hominis;* (Graczyk et al., 2007), from livestock, domestic and wild animals including rodents (*C. parvum*). *Cryptosporidium* has been detected in marine mammals (Hughes-Hanks et al., 2005; Rengifo-Herrera et al., 2010) although their infectiousness to humans is poorly understood, however *C.hominis* has been detected in a dougon in Australia (Morgan et al., 2000). Outbreaks associated with contaminated drinking water have been reported world-wide (Fayer et al., 2004; LeChevallier and Moser, 1995).

C. parvum oocysts can be concentrated by filter-feeding shellfish and survive for at least 30 days (Giangaspero et al., 2005; Graczyk et al., 2006; Graczyk et al., 2007; Guiguet Leal et al., 2008). Oocysts have been shown to survive for at least 12 months in seawater (Tamburrini and Pozio, 1999), but will be affected by temperature, salinity and amount of UV light (Nasser et al., 2007). The presence of *Cryptosporidium* oocysts will be dependent on the amount of faecal contamination, for example, in a site in Mexico oocysts where detected in more than 83 % of the samples at a concentration range of 150 to 2,050 oocysts/10 L (Magana-Ordorica et al., 2010). Because of the extreme persistence of *Cryptosporidium* oocysts in marine environments, these may persist in the absence of bacteria indicators of faecal contamination (Abdelzaher et al., 2010; Graczyk et al., 2010; Wilkes et al., 2011).

Inputs of *Giardia* cysts into marine environments can occur via freshwater (Fayer et al., 2004) from human sewage and bathers (both *G. duodenalis* and *G. enterica*), from livestock, dogs, cats and wild animals including rodents (*G. duodenalis*) and from dogs and some wild animals (*G. enterica*). *Giardia* has been detected in marine mammals (Hughes-Hanks et al., 2005) although their infectious to humans in poorly understood. Outbreaks associated with contaminated drinking water have been reported world-wide (LeChevallier and Moser, 1995).

Giardia cysts can be concentrated by filter-feeding shellfish and survive for at least 14 days (Graczyk et al., 2006; Graczyk et al., 1999). The presence of *Giardia* cysts will be dependent on the amount of faecal contamination, for example in a site in Mexico, cysts where detected in more than 70 % of the samples at a concentration range of 10-300 cysts/10 L (Magana-Ordorica et al., 2010).

Toxoplasma gondii oocysts can occur in both fresh and seawater (Lindsay et al., 2003). Inputs of oocysts into marine environments can occur via freshwater from the faeces of felines (Fayer et al., 2004) which can infect mammals living in the sea such as sea otters, dolphins and walruses (Conrad et al., 2005; Massie et al., 2010). Outbreaks associated with contaminated drinking water have been reported (Aramini et al., 1999; Bowie et al., 1997; de Moura et al., 2006).

T. gondii oocysts can be concentrated by filter-feeding shellfish and survive for several months (Lindsay et al., 2004; Lindsay et al., 2001). Survival can occur in filter-feeding fish (Northern anchovies and Pacific sardines) where oocysts persisted in their alimentary canals for at least 8h (Massie et al., 2010). Oocysts have been shown to survive for at least 6-24 months in seawater (Lindsay et al., 2003; Lindsay and Dubey, 2009).

Cryptosporidium, Giardia and Toxoplasma (oo)cysts will occur in animal faeces together with bacteria used as faecal indicators. However, because the protozoal (oo)cysts can persist longer than the indicators in marine environments, these may occur in the absence of detectable bacteria indicators of faecal contamination (Abdelzaher et al., 2010; Graczyk et al., 2010; Wilkes et al., 2011).

3.1.13.3. Adverse health effects and incidence

Within the genus *Cryptosporidium*, the major human pathogens are *Cryptosporidium parvum* and *Cryptosporidium hominis* which cause acute diarrhoea amongst the immunocompetent, particularly

amongst children under 5 years of age (Jex et al., 2011). Infections can be life-threatening in the immunocompromised, particularly those with AIDS.

Within the genus *Giardia*, the major human pathogens are *Giardia duodenalis* and *Giardia enterica* which cause a range of disease severity amongst the immunocompetent from asymptomatic infection to acute diarrhoea (Thompson, 2011).

Toxoplasmosis is widespread in humans and infection rates have been estimated to be 16-40 % in the UK and USA to 50-80 % in continental Europe, Central and South America. The majority of cases are sub-clinical amongst the immunocompetent, but there is increasing evidence that acquired toxoplasmosis can result in retinochoroiditis in a small proportion of infected persons (Gilbert and Stanford, 2000; Jones and Holland, 2010). Congenital infection can result in mild to serious disease (abortions and foetal deaths, retinochoroiditis, hydrocephalus, convulsions and intracerebral calcification) with lifelong disability (Dubey, 2011). Infections can be life-threatening in the immunocompromised, particularly those with AIDS where this parasite has been estimated to responsible for 10-30 % of deaths.

3.1.13.4. Conclusion on importance in seawater in comparison to other sources and possible inclusion in microbiological criteria

The cysts of protozoan parasites occur in aquatic environments as a result of faecal contamination (from both humans and other animals) and represent a hazard. However, contamination of seawater is probably of less importance than other routes of transmission. Since it is not possible, or technically difficult to grow these organisms in the laboratory, detection methods are difficult to perform, labour intensive and slow. Indicator organisms of faecal contamination are likely to provide an indication of recent faecal contamination, including the potential presence of pathogenic protozoa. Consequently this group of organisms is not further considered further in this document for inclusion in microbiological criteria.

3.2. Chemical hazards

EC Regulation 853/2004[9] requires the use of water of potable water quality by food business operators during food production. Criteria for the quality of potable water are laid down in Annex I, part B of Council Directive 98/83/EC[12] on the quality of water intended for human consumption (see Table 5, Appendix A, Current EU legislation) and in Commission Directive 2003/04/EC[13] establishing the list, concentration limits and labelling requirements for the constituents of natural mineral waters. Therefore a short description of the chemical hazards for the parameters included in these Directives is provided below. These chemical hazards are distinguished into a) inorganic chemicals and b) organic chemicals. Because it is stated in Council Directive 98/83/EC[12] that the standards in Annex I of this Directive are generally based on the World Health Organisation's 'Guidelines for drinking water quality', the respective WHO guideline values have also been included.

3.2.1. Inorganic chemicals

3.2.1.1. Antimony

Elementary antimony is found in alloys with copper, lead and tin. It is a normal raw water contaminant. The toxicity of antimony depends on its valency state. Most of the antimony leached from antimony containing materials would be in the form of antimony (V), which is the less toxic form. Although there is some evidence for the carcinogenicity of certain antimony compounds by inhalation, there are no data indicating that antimony might be carcinogenic by the oral route of exposure (WHO, 2011). The WHO established a Tolerable Daily Intake (TDI) for antimony of 6 µg/kg body weight (b.w.). Using an allocation of 10 % of the TDI to drinking water and assuming

[13] Commission Directive 2003/04/EC of the European Parliament and of the Council of 28 January 2003 on public access to environmental information and repealing Council Directive 90/313/EC. OJ L 41, 14.2.2003, p. 26-32.

consumption of two litres of drinking water by a 60 kg adult, a guideline value for antimony of 0.02 mg/l has been derived (WHO, 2004).

3.2.1.2. Arsenic

Arsenic is a metalloid that occurs in different inorganic and organic forms, which are found in the environment both from natural occurrence and from anthropogenic activity. Inorganic arsenic is more toxic as compared to organic arsenic. The Panel on Contaminants in the Food Chain recently evaluated the risks of arsenic in food (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009f). The main toxic effects reported to be associated with long term ingestion of inorganic arsenic in humans are skin lesions, and skin, lung and bladder cancer, effects that have been associated with high levels of arsenic in drinking water from wells in regions rich in arsenic in the earth's crust. Also acute neurotoxicity has been associated with high levels of arsenic in drinking water (well water). The International Agency for Research on Cancer (IARC) has classified arsenic as carcinogenic to humans (class I) (IARC, 2004). The CONTAM Panel noted that inorganic arsenic is not directly DNA-reactive and there are a number of proposed mechanisms of carcinogenicity such as oxidative stress, epigenetic effects and interference with DNA damage repair, for each of which a threshold mechanism could be postulated. The CONTAM Panel modelled the dose-response data from the key epidemiological studies and compared the outcome with the estimated dietary exposures to inorganic arsenic for average and high level consumers in Europe. Based on this assessment it was concluded that there is little or no margin of exposure and that the possibility of a risk to some consumers cannot be excluded. Therefore the CONTAM Panel recommended that dietary exposure to inorganic arsenic should be reduced.

The WHO (2004) derived a provisional guideline value for arsenic in drinking water of 0.01 mg/l. This guideline was designated provisional on the basis of treatment performance and analytical achievability.

3.2.1.3. Barium

Barium is present as a trace element in igneous and sedimentary rocks. Barium in water stems primarily from these natural sources. Food is the primary source of intake for the non-occupationally exposed population, but when concentrations of barium in water are high, drinking water may contribute considerably to the total intake. There is no evidence that barium is mutagenic or carcinogenic. The toxicological endpoint of concern to humans is its potency to raise blood pressure. Based on the most sensitive epidemiological study addressing this endpoint the WHO (2004) derived a guideline value of 0.7 mg/l.

3.2.1.4. Boron

Boron is naturally occurring in (ground) water as a result of leaching from rocks and soils containing borates and borosilicates. Oral toxicity studies in experimental animals have indicated that the developmental toxicity is the main endpoint. Boron is not genotoxic and long-term studies in rats and mice did not provide evidence for a carcinogenic potential. The Panel on Dietetic Products, Nutrition, and Allergies (NDA) has derived the following tolerable upper intake level (UL) values for boron: 10 mg boron/person/day for adults and 3, 4, 5, 7 and 9 mg boron/day for children aged 1-3, 4-6, 7-10, 11-14, and 15-17 years of age, respectively. These UL values apply to the intake of boron in the form of boric acid and borates (EFSA, 2004). The WHO (2009) established a TDI of 0.17 mg/kg b.w. and derived a guideline value of 2.4 mg/l (WHO, 2011).

3.2.1.5. Bromate

Bromate is not normally found in water but can occur as a result of ozonation when bromide ions are present in the water. Bromate is mutagenic, both *in vitro* and *in vivo* and it has been proposed that this might be due to oxidative DNA damage (WHO, 2011). There is inadequate evidence for carcinogenicity of bromate in humans, but bromate induced kidney tumours in experimental animals. Oxidative stress may play a role in the formation of renal tubule tumours, but current information is

insufficient to identify lipid peroxidation and production of reactive oxygen species (ROS) as key events in the induction of these tumours). IARC (1999a) has classified bromate in group 2B, possibly carcinogenic to humans. The WHO used the increased incidence of mesotheliomas, renal tubule tumours and thyroid follicular tumours, observed in a long-term drinking water study in rats, to derive a health-based guideline value for bromate in drinking water. Based on low-dose extrapolation the health based value associated with the upper-bound excess cancer risk of 10^{-5}, is 2 µg/l (WHO, 2004). Because of limitations in the available analytical and treatment methods the provisional WHO guideline value for bromate is 10 µg/l (WHO, 2004). This value has recently been reconfirmed (WHO, 2011).

3.2.1.6. Cadmium

Cadmium (Cd) is a heavy metal found as an environmental contaminant, both through natural occurrence and from industrial and agricultural sources. Foodstuffs are the main source of cadmium exposure for the non-smoking general population. Cadmium has recently been evaluated by the CONTAM Panel (EFSA, 2009e). It is primarily toxic to the kidney, especially to the proximal tubular cells where it accumulates over time and may cause renal dysfunction. Beta-2-microglobulin (B2M), a low molecular weight protein which is found in urine, is recognised as the most useful biomarker in relation to tubular effects.

Cadmium levels in urine are widely accepted as a measure of the body burden of Cd and the cumulative amount in the kidneys. The CONTAM Panel carried out a meta-analysis on a large set of epidemiological studies to evaluate the dose-response relationship between urinary cadmium and urinary B2M. A mathematic model was fitted to the dose-response relationship between urinary cadmium and B2M for subjects over 50 years of age and for the whole population. From the model, a benchmark dose lower confidence limit for a 5 percent increase of the prevalence of elevated B2M ($BMDL_5$) of 4 µg Cd/g creatinine was derived. A chemical-specific adjustment factor of 3.9, to account for inter-individual variation of urinary cadmium within the study populations, was applied, leading to a value of 1.0 µg Cd/g creatinine.

The CONTAM Panel estimated that the dietary Cd exposure that corresponds to the critical urinary cadmium concentration of 1 µg/g creatinine after 50 years of exposure was 0.36 µg Cd/kg b.w., corresponding to a weekly dietary intake of 2.52 µg Cd/kg b.w. The mean exposure for adults across Europe is close to, or slightly exceeding, the TWI of 2.5 µg/kg b.w. Although the risk for adverse effects on kidney function at an individual level at dietary exposures across Europe is very low, the CONTAM Panel concluded that the current exposure to Cd at the population level should be reduced (EFSA, 2009e).

In 2004, the WHO used a provisional tolerable weekly intake (PTWI) of 7 µg/kg b.w., an allocation of 10 % of the PTWI, and a consumption of two litres of drinking water per day for a 60 kg adult, to derive a guideline value for cadmium in drinking water of 0.003 mg/l (WHO, 2004). This value was reconfirmed in 2011 (WHO, 2011).

3.2.1.7. Chromium

Chromium is a widely distributed natural element. It can exist in different valences of +2 to +6 (chromium II to VI). Chromium III is an essential element. The toxicological database of chromium is limited. Chromium III is not genotoxic, but chromium VI is genotoxic in a wide range of *in vitro* and in *vivo tests* (WHO, 2011). In a long-term oral carcinogenicity study in rats given chromium III, no increase in tumour incidence was observed. Chromium VI is carcinogenic following inhalation exposure. IARC (1990, 2011) has classified chromium VI in group I (human carcinogen) and chromium III in group 3 (not classifiable as to its carcinogenicity to humans). It should be noted that chromium VI is reduced to chromium III in the stomach and the gastrointestinal tract (WHO, 2011). As a 'practical measure' a provisional guideline value of 0.05 mg/l has been derived by WHO (2004) for total chromium.

3.2.1.8. Copper

Copper is a widely distributed natural element and an essential nutrient. It serves as co-factor for many important metalloproteins such as cytochrome oxidase, copper-zinc dismutase, ceruloplasmin and tyronase. Food and drinking water are the primary sources of exposure of humans. However, high copper intake may lead to copper toxicosis primarily affecting the liver, the kidneys and the gastrointestinal tract as indicated in an external report to EFSA.[14] The most sensitive acute adverse effects of copper are gastrointestinal effects (WHO, 2004, 2011). These effects formed the basis for the derivation of the guideline value of 2 mg/l by the WHO to protect people with a normal copper homeostasis against acute gastrointestinal effects. It was however noted that there are uncertainties regarding the long-term effects in sensitive populations, such as patients suffering from Wilson disease or other metabolic disorders (WHO, 2004, 2011).

3.2.1.9. Cyanide

Cyanide is only occasionally found in drinking water but usually at very low concentrations (WHO, 2011). Cyanide is highly acutely toxic. Oral lethal doses for cyanide expressed as CN^- in rats and dogs are 4-6 and 2 mg/kg b.w., respectively (EFSA, 2007). Cyanide ion (CN^-) binds to cytochrome oxidases in the mitochondria, thereby inhibiting the intracellular oxidative processes. This leads to death through hypoxia. The organ that is most sensitive to cyanide toxicity is the brain. According to the WHO (2011) data on acute toxicity are unsuitable for deriving a health-based value for short-term exposure.

Based on effects on reproductive organs in a 13-week oral toxicity study a TDI of 0.045 mg/kg b.w. was established. Allocating 40 % of the TDI to drinking water and assuming a 60 kg adult drinking two litres of water per day, a health based value of 0.5 mg/l (rounded value) for short-term exposure was derived (WHO, 2011). It was noted that the lowest reported odour threshold for cyanide in drinking water of 0.17 mg/l was below this health-based value. Since cyanide concentrations in drinking water are 'well below those of health concern' it was concluded that derivation of a formal guideline value for short-term exposure was not necessary (WHO, 2011).

3.2.1.10. Fluoride

Fluorides are widely distributed in earth's crust and can be found in minerals such as fluorspar, cryolite and fluorapatite. Fluoride might be an essential element for humans, although this has not been demonstrated unequivocally. There is evidence that fluoride plays a role in the prevention of dental caries. Epidemiological studies with fluoride in drinking water has indicated that the minimum concentration to produce these protective effects is about 0.5 mg/l, and that the degree of protection may increase up to concentrations of 2 mg/l. The therapeutic margin is however small, since, depending on the amount of drinking water consumed, mild dental fluorosis can be observed from about 1 mg/l onwards. More serious effects such as skeletal fluorosis may be observed at concentrations in the range of 3-6 mg fluoride/l. Available studies indicate that the evidence for a carcinogenic potential of fluoride in experimental animals is inconclusive and that there is no evidence for carcinogenicity in humans. Overall, the WHO concluded that there was no reason to revise the previously derived guideline value of 1.5 mg/l (WHO, 2011).

The NDA Panel derived the following tolerable upper intake level (UL) values: 7 mg fluoride per day for the population of 15 years and older (adults) and 5 mg fluoride per day for children of 9 to 14 years of age, based on the most critical endpoint, bone fracture. An UL of 1.4 mg fluoride per day was established for children of 1 to 3 years of age and 2.2 mg fluoride per day for children of 4 to 8 years of age, based on the critical endpoint, moderate dental fluorosis (EFSA, 2005a).

[14] External scientific report: Selected trace and ultratrace elements: Biological role, content in feed and requirements in animal nutrition – Elements for risk assessment. Available online: www.efsa.europa.eu/en/supporting/doc/68e.pdf

3.2.1.11. Lead

Lead is an environmental contaminant that occurs naturally and, to a greater extent, from anthropogenic activities such as mining and smelting and battery manufacturing. Lead is a metal that occurs in organic and inorganic forms; the latter predominates in the environment. Human exposure to lead can occur via food, water, air, soil and dust. Food is the major source of lead exposure. Lead is rarely present in drinking water as a result of its dissolution from natural sources and concentrations in drinking water are usually below 5 mg/l (WHO, 2011). Lead has recently been evaluated by the CONTAM Panel (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2010a).

Due to its long half-life in the body, chronic toxicity of lead is of most concern when considering the potential risk to human health. Studies with rodent and non-human primate models have demonstrated that chronic low-level exposure to lead causes neurotoxicity, particularly learning deficits in the developing animal.

At current exposure, the central nervous system is considered to be the main target organ for lead toxicity in humans, and there is considerable evidence demonstrating that the developing brain is more vulnerable to the neurotoxicity of lead than the mature brain. In addition, several studies identified an association between blood lead concentration, elevated systolic blood pressure (SBP) and chronic kidney disease (CKD), at relatively low blood lead (B-Pb) levels (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2010a).

Lead may be a weak indirect genotoxic metal. There is extensive experimental evidence that at high doses lead can induce tumours at a number of different sites in rodents. The IARC (2006) has classified inorganic lead as probably carcinogenic to humans (Group 2A).

In humans, the central nervous system is the main target organ for lead toxicity, and there is considerable evidence demonstrating that the developing brain is more vulnerable to the neurotoxicity of lead than the mature brain. In addition, several studies identified an association between blood lead concentration, elevated SBP and CKD, at relatively low B-Pb levels.

The CONTAM Panel identified developmental neurotoxicity in young children and cardiovascular effects and nephrotoxicity in adults as potential critical adverse effects of lead on which to base the risk assessment. Full Scale IQ score was identified as the most relevant endpoint for children. Dose-response analysis of cardiovascular effects and nephrotoxicty identified effects on systolic blood pressure and effects on glomerular filtration rate as the most critical. These endpoints were assessed using B-Pb as the most appropriate dose metric. The resulting B-Pb level associated with the 95^{th} lower confidence limit of the benchmark dose (BMD) of 1 % extra risk ($BMDL_{01}$) for the respective endpoint were then converted by mathematical modelling into the respective dietary exposure. For children a $BMDL_{01}$ dietary intake value of 0.50 µg/kg b.w. per day for developmental neurotoxicity was derived. For adults the $BMDL_{01}$ dietary lead intake values for cardiovascular and kidney effects were 1.50 µg/kg b.w. per day and 0.63 µg/kg b.w. per day, respectively. Since these values are all below the PTWI of 25 µg/kg b.w. set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO, 1986, 2000) and endorsed by the Scientific Committee of Food (SCF, 1992), the CONTAM Panel concluded that this PTWI is no longer appropriate.

As there was no evidence for a threshold for the key effects of lead, The CONTAM Panel concluded that it would not be appropriate to derive a PTWI, and therefore used a margin of exposure (MOE) approach in its risk characterization. Although the MOE is small it was concluded that the risk of clinically important effects on either the cardiovascular system or kidneys of adult consumers, at current levels of lead exposure is low to negligible. In infants, children and pregnant women, there is potential concern at current dietary levels of exposure to lead for effects on neurodevelopment. Therefore it was recommended to continue reduction of exposure to lead (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2010a).

The WHO (2004) derived a provisional guideline value for lead in drinking water of 0.01 mg/l, based on a PTWI of 25 µg/kg b.w., an allocation of 50 % and a drinking water consumption of 0.75 l for a 5 kg infant. This guideline was designated provisional on the basis of treatment performance and analytical achievability (WHO, 2004, 2011).

3.2.1.12. Manganese

Manganese is one of the most abundant metals. It is naturally occurring in many surface water and groundwater sources. Manganese is an essential element that functions as an enzyme activator and is a constituent of several enzymes such as glycosyltransferase, pyruvate carboxylase and manganese dismutase as indicated in an external report to EFSA.[15] Conflicting results on effects of long-term exposure to high levels of manganese in drinking water have been reported in epidemiological studies. Studies showing adverse neurological effects have significant confounders, and a number of other studies did not show any effect. Results from toxicological studies with experimental animals, particularly in rodents, are not an appropriate basis for human risk assessment, because the physiological requirements for manganese vary among different animal species (WHO, 2011).

The WHO (1996, 2004) derived a health-based value of 0.4 mg/l, but because this value is well above the manganese concentration normally found in drinking water it was not considered necessary to derive a formal guideline value (WHO, 2011).

3.2.1.13. Mercury

Inorganic mercury in uncontaminated drinking water is usually in the form of Hg^{2+}. In fresh water and seawater methylation of inorganic mercury may occur. Nephrotoxicity is the most sensitive endpoint following chronic ingestion of inorganic mercury. Mercury II has the potential to increase the incidence of benign tumours at sites where tissue damage is apparent. It also exhibits weak genotoxic activity but does not induce point mutations (WHO, 2011). IARC (1993) classified metallic mercury and inorganic mercury compounds as not classifiable as to their carcinogenicity to humans (Group 3).

Based on kidney effects observed in long-term toxicity experiments in rats the WHO established a TDI of 2 µg/kg b.w. Allocating 10 % of the TDI to drinking water and assuming consumption of two litres of drinking water by a 60 kg adult, a guideline value for inorganic mercury of 6 µg/l has been derived (WHO, 2004). This value has recently been reconfirmed (WHO, 2011).

3.2.1.14. Nickel

Food is the major source of exposure of the non-smoking, non-occupationally exposed human population. Water generally is a minor contributor to daily oral exposure, with nickel concentrations in drinking water usually below 0.02 mg/l. Nickel is generally not accepted as an essential nutrient for higher animals, apparently because of the lack of a clearly defined specific biochemical function and no enzymes or co-factors are known that include nickel in higher organisms. EFSA (2005b) considers the essentiality of nickel for humans to be not demonstrated.

Nickel compounds do not show mutagenic activity in bacterial tests, but show weak positive results in cultured mammalian cells tested for chromosomal aberrations. Also a weak increase in sister chromatid exchange, disturbances of spindle function, the inhibition of DNA synthesis / repair and the induction of cell transformation have been observed in *in vitro* tests with nickel compounds (EFSA, 2005b). IARC (1990) evaluated the carcinogenic risks of nickel and classified nickel compounds as carcinogenic to humans (Group 1) and metallic nickel as possibly carcinogenic to humans (Group 2B). Overall, the toxicological database for oral exposure to nickel is limited and EFSA (2005b) considered the available animal studies inadequate to identify a non-observed-adverse-effect level (NOAEL).

[15] External scientific report: Selected trace and ultratrace elements: Biological role, content in feed and requirements in animal nutrition – Elements for risk assessment. Available online: www.efsa.europa.eu/en/supporting/doc/68e.pdf

The WHO established a TDI of 12 µg/kg b.w. based on effects after oral provocation of fasted patients with dermal nickel allergy. Using an allocation of 20 % of the TDI to drinking water and assuming consumption of two litres of drinking water by a 60 kg adult, a guideline value for nickel of 70 µg/l has been derived (WHO, 2004).

3.2.1.15. Nitrate

Nitrate is a naturally occurring compound that is part of the nitrogen cycle, as well as an approved food additive. It plays an important role in the nutrition and function of plants. Nitrate can reach both surface and groundwater as a result of agricultural activity. In general, the most important source of human exposure to nitrate is through consumption of vegetables, and to a lesser extent through water and other foods. In the case of bottle-fed infants drinking water can be a major source of exposure to nitrate (EFSA, 2008a; WHO, 2011). Nitrate *per se i*s relatively non-toxic, but its metabolites and reaction products e.g., nitrite, nitric oxide and N-nitroso compounds, have raised concern because of implications for adverse health effects such as methaemoglobinaemia and carcinogenesis.

In humans methaemoglobinaemia is a consequence of the reaction of nitrite with haemoglobin to form methaemoglobin which binds oxygen tightly and does not release it, thereby blocking oxygen transport. Particularly bottle-fed infants could be at risk due to their high intake in relation to their bodyweight and the limited presence of enzymes to convert methaemoglobin back to haemoglobin. There is compelling evidence that the risk of methaemoblobinaemia is primarily increased in the presence of simultaneous gastrointestinal infections (WHO, 2011). On the other hand recent research indicates that nitrite participates in host defence having antimicrobial activity, and other nitrate metabolites e.g. nitric oxide, have important physiological roles such as vasoregulation. Epidemiological studies do not indicate that nitrate intake from diet or drinking water is associated with increased cancer risk (EFSA, 2008a).

The former EU Scientific Committee on Food (SCF) reviewed the toxicological effects of nitrate and established an acceptable daily intake (ADI) of 0-3.7 mg/kg b.w. based on growth reduction observed in a chronic toxicity study (EC, 1992). The more recent assessment of nitrate by the Joint Expert Committee on Food Additives (JECFA) (FAO/WHO, 2003) reconfirmed this ADI. In the evaluation of nitrate the CONTAM Panel concluded that in the absence of significant new toxicological and toxicokinetic data, there was no need to re-consider this ADI (EFSA, 2008a).

The WHO reconfirmed the guideline value of 50 mg/l for nitrate (or 11 mg/l as nitrate nitrogen) to protect against methaemoglobinaemia in bottle-fed infants (WHO, 2011).

3.2.1.16. Nitrite

Nitrite is formed naturally by the nitrogen cycle during the process of nitrogen fixation and it is subsequently converted to nitrate, a major nutrient assimilated by plants. Nitrite is formed in nature by the action of nitrifying bacteria as an intermediate stage in the formation of nitrates. Conversely, conversion of nitrate to nitrite and other metabolites (nitric oxide and N-nitrosocompounds) may occur either in the saliva of most monogastric animals or in the stomach of ruminants due to microbiological action. This nitrite formation is the origin of the acute toxicity of nitrate (methaemoglobinaemia). The acute toxicity of nitrite is about 10 times higher than that of nitrate (EFSA, 2008a, 2009a).

In a long-term carcinogenicity study there was equivocal evidence for carcinogenic activity in female mice based on the combined incidence of squamous cell papilloma and carcinoma of the forestomach (NTP, 2001). In view of the lack of genotoxicity of nitrite, the absence of tumours in rats and male mice, and the fact that humans do not possess a forestomach, the relevance of this observation is doubtful (WHO, 2011).

The SCF reviewed the toxicological effects of nitrite and established an ADI of 0-0.06 mg/kg based on heart and lung toxicity in a long-term study in rats (EC, 1997). The JECFA (2002) established an ADI of 0-0.07 mg/kg b.w. for nitrite based on the same data. The latter figure does not differ significantly

from the ADI established by the SCF. In the absence of significant new toxicological and toxicokinetic data, the CONTAM Panel concluded that there was no need to re-consider this ADI (EFSA, 2008a, 2009a).

The WHO reconfirmed the guideline value of 3 mg/l for nitrite (or 0.9 mg/l as nitrite nitrogen) to protect against methaemoglobinaemia in bottle-fed infants (WHO, 2011).

3.2.1.17. Selenium

Selenium is present in the earth's crust in association with sulphur-containing minerals. Selenium is an essential element the biological functions of which are mediated via specific selenoproteins/ selenoenzymes, hydrogen selenide and methylated selenium compounds, respectively. A large number of selenoproteins has been identified, virtually all containing selenocysteine. The most important selenoproteins are peroxidases, deiodinases, and thioredoxin reductase (EFSA, 2006).

The toxicological database for selenium is limited. A short term (28 day) toxicity study in Wistar rats revealed that the oral administration of 1000 µg Se/kg b.w. per day resulted in reduced weight gain and food consumption, and induced hepatotoxicity, including vacuolization and necrosis of hepatocytes, increased apoptosis and acute inflammation. No NOAEL was identified (EFSA, 2008b). Selenium compounds (i.e., selenite, selenate, selenide, selenocysteine, selenosulphide) showed a moderate genotoxic activity in several *in vitro* tests. These studies indicate that the mutagenic effects of selenium salts are associated with the production of ROS. It is well known that auto-oxidisable selenium metabolites, such as hydrogen selenide, can undergo redox cycling producing oxygen radicals and cause DNA strand breaks (SCF, 2000).

In 2011 the WHO replaced the previously derived guideline value of 0.01 mg/l (WHO, 1996, 2004) by a provisional guideline value of 0.04 mg/l, based on an allocation of 20 % to the upper tolerable intake of 400 µg/person per day established by FAO/WHO (2004) and a daily consumption of two litres drinking water. This guideline value is designated as provisional because of the uncertainties inherent in the scientific database (WHO, 2011).

3.2.2. Organic chemicals

3.2.2.1. Acrylamide

Acrylamide may be formed in carbohydrate-rich and protein-low food commodities, during cooking or other thermal processing such as frying, baking or roasting at temperatures of 120 °C or higher (EFSA, 2005c). In drinking water acrylamide monomer may be present due to the use of polyacrylamide coagulants used in drinking water treatment. In seawater acrylamide is usually not detectable (WHO, 2011). Acrylamide is neurotoxic, affects germ cells and impairs reproductive function. In long-term animal studies with rodents acryl amide administered in the drinking water was carcinogenic (WHO, 2011). The IARC classified acrylamide as probably carcinogenic to humans, class 2A (IARC, 1994). Acrylamide was negative in bacterial mutagenicity assays but it induced gene mutations in mammalian cells and chromosal aberration *in vitro* and *in vivo*. Based on the combined incidence of mammary, thyroid and uterine tumours observed in female rats in a long-term drinking-water study, and using the linearized multistage model the WHO (2004) derived a guideline value for acrylamide of 0.0005 mg/l (0.5 µg/l).

3.2.2.2. Benzene

Benzene may be present in water resulting from industrial effluents and atmospheric pollution. In humans acute occupational exposure to high levels of benzene might cause effects on the central nervous system. Benzene causes leukaemia in humans and experimental animals. It is not mutagenic in bacterial assays but induces chromosomal aberrations in vivo in a number of species including humans. The WHO (2004) has derived a guideline value for benzene of 0.01 mg/l, based on linear extrapolation applied to the incidence of leukaemia and lymphomas in female mice and oral cavity squamous cell carcinomas in male rats in two-year gavage studies.

3.2.2.3. Benzo(a)pyrene and other polycyclic aromatic hydrocarbons

Polynuclear aromatic hydrocarbons (PAHs) form a large class of diverse organic compounds composed of two or more fused aromatic rings of carbon and hydrogen atoms. Most PAHs are emitted into the environment from a variety of combustion and pyrolysis processes. Due to their low water solubility and high affinity for particulate matter PAHs are usually not detected in water in notable concentrations (WHO, 2011). If detected in drinking water the origin usually is the coal-tar coating of distribution pipes. This situation does not occur for clean seawater.

The SCF (EC, 2002) concluded that 15 PAHs, namely benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, cyclopenta[*cd*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[1,2,3-*cd*]pyrene and 5-methylchrysene show clear evidence of mutagenicity/genotoxicity in somatic cells in experimental animals. With the exception of benzo[*ghi*]perylene they have also shown clear carcinogenic effects in experimental animals in bioassays using various (non-oral) routes of exposure. According to the SCF these compounds may be regarded as potentially carcinogenic to humans and therefore present a health concern. Only for benzo(a)pyrene there is evidence for carcinogenicity following oral administration. Therefore benzo[*a*]pyrene has often been used as indicator to estimate the risk of exposure to PAHs. Based on an increase in gastric tumours in mice administered benzo[*a*]pyrene in the diet and applying linearized multistage extrapolation, the WHO (1996) derived a guideline value for benzo(a)pyrene of 0.7 µg/l, associated with an excess cancer risk of 10^{-5}. This guideline value has since then been retained (WHO, 2011). However, in the drinking water domain also other PAHs such as benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, and indeno(1,2,3-cd)pyrene have been proposed as parameters to be monitored (see Annex A, Chapter 6).

3.2.2.4. 1,2-Dichloroethane

1,2-Dichloroethane may enter surface water via industrial effluents and ground water following leaching from waste disposal sites. In orally exposed experimental animals 1,2-dichloroethane affects the immune system, the central nervous system, the liver and the kidney. 1,2-Dichloromethane is mutagenic in bacterial assays and mammalian cells. It induces DNA damage in liver cells *in vivo* and binds to DNA, RNA and proteins in animals. IARC (1999b) classified 1,2-dichloroethane as possibly carcinogenic to humans (Group 2B). By applying the linearized multistage model to the incidence of haemangiosarcomas in rats the WHO (2004) derived a guideline value of 0.03 mg/l.

3.2.2.5. Epichlorohydrin

Epichlorohydrin is used for the manufacture of water treatment coagulant polymers. No quantitative data are available on its occurrence in drinking water, but it has been noted that it is slowly hydrolysed in aqueous media (WHO, 2011). In long-term studies epichlorohydrin induces tumours of the forestomach in rodents following oral exposure. Based on information from occupationally exposed humans the IARC (1999b) classified epichlorohydrin in group 2A, probably carcinogenic to humans. The WHO (2004) established a TDI of 0.14 µg/kg b.w. for epichlorohydrin based on forestomach hyperplasia and using an uncertainty factor of 10,000: 100 for inter- and intraspecies variation, 10 for the use of a LOAEL instead of a NOAEL, and 10 for carcinogenicity. The use of the linearized multistage model for estimating cancer risk was considered inappropriate by WHO, because tumours were seen only at the site of administration, where epichlorohydrin is highly irritating. Allocating 10 % of this TDI to drinking water and assuming a drinking water consumption of 2 l/day by a 60 kg adult, a provisional guideline value of 0.4 µg/l was derived. This guideline value was considered provisional because of the uncertainties regarding the toxicity of epichlorohydrin and the large uncertainty factor used in establishing the TDI (WHO, 2004).

3.2.2.6. Pesticides

Pesticides (plant protection products) form a large class of compounds comprising acaricides, algicides, biocides, fungicides, herbicides, insecticides, nematocides, rodenticides and slimicides. Over

the years several international organisations such as the Joint FAO/WHO meeting on Pesticides Residues (JMPR), the EU Scientific Committee on Food and EFSA have evaluated pesticides and established an Acute Reference Dose for pesticides with a prominent acute toxicity such as organophosphates and carbamates, and an Acceptable Daily Intake for pesticides exerting toxic effects following long-term exposure. The WHO has derived health based guideline values for a wide range of pesticides, an overview of which is presented in the Fourth Edition of 'Guidelines for Drinking-Water Quality' (WHO, 2011). In Directive 98/83/EC[12] (see Table 9, Annex A) the European Commission has set a low parameter value for pesticides (0.1 µg/l for individual pesticides and 0.5 µg/l for Total) actually implying the need for absence of pesticides in drinking water.

3.2.2.7. Tetrachloroethene

Tetrachloroethene is widespread in the environment and can be found in trace amounts in water. Industrial emissions can sometimes lead to high levels in groundwater. The major toxic effects of long-term exposure to tetrachloroethene are on the liver and the kidney. The overall evidence indicates that it is not genotoxic (WHO, 2011). The IARC (1999b) classified it as group 2A (probably carcinogenic to humans). Based on the NOAEL for liver toxicity in male mice and male and female rats in repeated dose toxicity studies the WHO established a TDI of 14 µg/kg b.w. Allocating 10 % of the TDI to drinking water and assuming that a 60 kg adult consumes two litres per day a guideline value of 0.04 mg/l was derived (WHO, 2004).

3.2.2.8. Trichloroethene

Trichloroethene is mainly emitted to the atmosphere, but can also leach into groundwater and to a lesser extent into surface water. Trichloroethene appears to be weakly genotoxic *in vitro* and *in vivo*. Although it is carcinogenic in rodents the WHO (2004) concluded that the critical effect is developmental toxicity. The IARC (1999b) classified trichloroethene as group 2A (probably carcinogenic to humans). Based on a $BMDL_{10}$ for developmental effects in rats the WHO established a TDI of 1.46 µg/kg b.w. Allocating 50 % of the TDI to drinking water and assuming that a 60 kg adult consumes two litres per day a guideline value of 0.02 mg/l was derived (WHO, 2004).

3.2.2.9. Trihalomethanes

Chlorination of raw water results in the formation of trihalomethanes. Chloroform is the most common disinfection by-product, but also bromoform, bromodichloromethane and dibromochloromethane can be formed (WHO, 2011).

Chloroform causes liver toxicity and in long-term studies in mice liver tumours have been observed. The weight of evidence indicates that chloroform is not genotoxic. The IARC (1999c) classified chloroform as possibly carcinogenic to humans (group 2B). Based on a threshold mechanism for liver carcinogenicity the WHO established a TDI of 15 µg/kg b.w. Allocating 75 % of the TDI to drinking water and assuming that a 60 kg adult consumes 2L/day a guideline value of 0.3 mg/l was derived (WHO, 2004).

Data from a variety of assays on the genotoxicity of bromoform are equivocal. It did not induce tumours in mice but a small increase in tumours of the large intestine has been observed in rats (WHO, 2011). The IARC (1999b) classified bromoform in group 3 (not classifiable as to its carcinogenicity to humans). Based on the NOAEL for liver toxicity in a well-conducted 90-day study in rats the WHO (2005) established a TDI for bromoform of 18 µg/kg b.w. Allocating 20 % of the TDI to drinking water and assuming that a 60 kg adult consumers two litres per day a guideline value of 0.1 mg/l was derived (WHO, 2004).

Genotoxicity data for dibromochloromethane are inconclusive. It causes liver toxicity in rats and mice. In long-term toxicity studies it induced hepatic tumours in female mice but not in rats (WHO, 2011). The IARC (1999b) classified dibromochloromethane in group 3 (not classifiable as to its carcinogenicity to humans). Based on the NOAEL for liver toxicity in a well-conducted 90-day study in rats the WHO (2005) established a TDI for dibromochloromethane of 21 µg/kg b.w. Allocating

20 % of the TDI to drinking water and assuming that a 60 kg adult consumes two litres per day a guideline value of 0.1 mg/l was derived (WHO, 2004).

Bromodichloromethane gave inconclusive results in a variety of *in vitro* and *in vivo* genotoxicity assays. Conflicting results have been observed with respect to the carcinogenicity of bromodichloromethane in long-term studies in rats and mice (WHO, 2011). The IARC (1999b) has classified it in group 2B (possibly carcinogenic to humans). The WHO (2011) has derived a guideline value of 0.06 mg/l drinking water.

3.2.2.10. Vinyl chloride

Due to its high volatility vinyl chloride has rarely been detected in surface water. In groundwater it can be found as a degradation product of tri- and tetrachloroethene. Metabolites of vinyl chloride are genotoxic producing DNA adducts. In occupationally exposed humans chromosomal aberrations, micronuclei and sister chromatid exchanges have been found, confirming the genotoxicity of vinyl chloride in vivo. Based on the carcinogenicity of vinyl chloride in workers exposed via inhalation the IARC (2008) has classified vinyl chloride in group 1 (carcinogenic to humans). Following oral administration of vinyl chloride to rodents, tumours in the mammary gland, Zymbal gland, the liver and other sites have been observed. Linear extrapolation from the dose causing tumours in 10 % of the rats, and assuming a doubling of the risk for exposure from birth, resulted in a guideline value of 0.3 µg/l, associated with the upper-bound excess cancer risk of 10^{-5} (WHO, 2004).

3.2.3. Occurrence of chemicals in seawater

A large database on levels of chemicals in seawater has been made available by the International Council for the Exploration of the Sea (ICES). It contains concentration data from samples of seawater collected at various locations in the North Sea and in the coastal waters of Denmark (both at the West and East coast), the Baltic Sea and from the Atlantic Ocean (ICES, 2012). Another study provided information on levels of a number of compounds in seawater from the North Sea and the North-eastern Atlantic (AFSSA, 2006). The results are presented in Table 1.

Table 1: Concentrations of chemicals in seawater (µg/l)

	Number of samples	Mean	Range	Reference
Inorganics				
Antimony	n.r.[a]	0.18– 0.22	n.d[b].	Filella et al., 2002
Arsenic	154	1.6	0.4 – 4.8	ICES, 2012
Barium	n.r.	6	n.r.	WHO, 1990
Boron	19	3600	700 - 4900	ICES, 2012
Bromate	n.d.	n.d.	n.d.	
Cadmium	383	0.07	0.005 – 1.3	ICES, 2012
	n.r.		0.005 – 0.025	AFFSA, 2006
Chromium	241	1.5	0.2 – 6.3	ICES, 2012
Copper	384	1.8	0.21 – 6.1	ICES, 2012
	n.r.	n.r.	0.05 – 0.36	AFFSA, 2006
Cyanide	n.d.	n.d.	n.d.	
Fluoride	n.r.	.n.r.	1200 – 1500	Camargo, 2003
Lead	393	0.8	0.001 – 7.6	ICES, 2012
	n.r.	n.r.	0.005 – 0.02	AFFSA, 2006
Manganese	29	6.2	0.7- 10.8	ICES, 2012
Mercury	673	0.012	0.00014 – 0.09	ICES, 2012
	n.r.	n.r.	0.0001 – 0.0005	AFFSA, 2006
Nickel	237	1.1	0.31 – 3.9	ICES, 2012
Nitrate	6583	78	1 - 4400	ICES, 2012
Nitrite	9255	2.4	0.2 – 94	ICES, 2012
Selenium	n.r.	n.r.	0.01 – 0.45	Nagpal and Howell, 2001
Organics				
Acrylamide	n.d.	n.d.	n.d.	
Benzene	57	0.75	0.05 – 1.5	ICES, 2012
Benzo(a)pyrene	367	0.00029	0.000003- 0.012	ICES, 2012
	-		0.000001 – 0.000005	AFFSA, 2006
1.2-Dichloroethane	95	0.8	0.1 - 2	ICES, 2012
Epichlorohydrin	n.d.	n.d.	n.d.	
Pesticides	n.d.	n.d.	n.d.	
Tetrachloroethene	-	0.012	0.0002 – 2.6	De Raat, 2003
Trichloroethene	97	0.09	0.05 – 0.2	ICES, 2012
Trihalomethanes[(c)]	93	0.12	0.05 – 0.8	ICES, 2012
Vinyl chloride	n.d.	n.d.	n.d.	

(a): n.r. means not reported
(b): n.d. means no data identified
(c): Data only for chloroform

For most of the chemicals addressed above information on the concentration in seawater could be identified. The data in Table 1 show that mean concentrations are usually in the low µg/l range or even lower. For boron, however, the mean concentration is 3.6 mg/l (range 0.7-4.9 mg/l). In contrast, for benzo(a)pyrene the mean concentration is about 0.3 ng/l. However, this concentration can be higher locally due to e.g. oil spills.

For a number of organic compounds such as acrylamide, epichlorohydrin and vinyl chloride no data are available. Since these compounds are used in drinking water treatment and transport they were

included as parameter in the drinking water directive (Council Directive 98/83/EC[12]), but concentrations in seawater can be expected to be low.

Other organic contaminants such as polychlorinated biphenyls (PCBs), dioxins, brominated flame retardants and organotin antifouling agents have raised concern regarding contamination of marine life. Because of their lipophylic properties they are bioaccumulative and higher concentrations are found higher in the marine food chain, e.g. in fish. Because these compounds have a low solubility in water their concentrations in sea water are in general very low. For PCBs, for instance, the concentration in seawater is in the order of pg/l (AFSSA, 2006). Therefore these contaminants are not considered to be a potential hazard with respect to the use of clean seawater.

3.3. Phytoplankton/algae

Algae may be present in seawater, particularly in coastal waters and estuaries. A number of marine algae species such dinoflagellates and diatoms have been reported to be associated with toxic syndromes in humans known as diarrhoeic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), paralytic shellfish poisoning (PSP) and neurotoxic shellfish poisoning (NSP). Outbreaks of these toxic syndromes were primarily found in humans that consumed filter-feeding bivalve molluscs such as oysters, mussels, scallops, and clams, that had concentrated the toxins present in the algae in their digestive gland (hepatopancreas). EFSA has released a series of opinions on marine biotoxins that could be present in bivalve molluscs. The most important toxins related to human intoxications are described below.

Diarrhoeic Shellfish Poisoning (DSP) is caused by okadaic acid (OA)-group toxins. These toxins are usually produced by dinoflagellates that belong to the genera *Dinophysis* spp. and *Prorocentrum* spp. DSP is characterized by symptoms such as diarrhoea, nausea, vomiting and abdominal pain, and is found in humans, shortly after ingestion of contaminated bivalve molluscs (EFSA, 2008c).

Amnesic shellfish poisoning (ASP) in humans is caused by domoic acid (DA) which is present mainly in marine red algae of the genus *Chondria* and diatoms of the genus *Pseudo-nitschia*. Symptoms of ASP include gastrointestinal effects (nausea, vomiting, diarrhoea or abdominal cramps) within 24 hours of consuming shellfish contaminated with DA and/or neurological symptoms or signs (confusion, loss of memory, or other serious signs such as seizure or coma) occurring within 48 hours (EFSA, 2009b).

Paralytic shellfish poisoning (PSP) in humans is caused by saxitoxin (STX)-group toxins. Symptoms of human PSP intoxication vary from a slight tingling sensation or numbness around the lips to fatal respiratory paralysis. Fatal respiratory paralysis occurs 2 to 12 hours following consumption of shellfish contaminated with STX-group toxins. STX-group toxins are mainly produced by dinoflagellates belonging to the genus *Alexandrium*: e.g. *Alexandrium tamarensis*, *A.minutum* (syn. *A. excavata*), *A. catenella*, *A. fraterculus*, *A. fundyense* and *A. cohorticula*. Also other dinoflagellates such as *Pyrodinium bahamense* and *Gymnodinium catenatum* have been identified as sources of STX-group toxins (FAO, 2004). Shellfish feeding on these algae can accumulate the toxins, but the shellfish itself is rather resistant to the harmful effects. STX-producing algae species occur worldwide, both in tropical and moderate climate zones (EFSA, 2009c).

Neurotoxic shellfish poisoning (NSP) is characterised by mainly neurological and gastrointestinal effects including e.g. nausea, vomiting, diarrhoea, parasthesia, cramps, bronchoconstriction, paralysis, seizures and coma. These effects are caused by brevetoxin (BTX) group toxins which are primarily produced by a dinoflagellate *Karenia brevis*. To date, NSP appears to have been limited to the Gulf of Mexico, the east coast of the United States of America (U.S.A.), and the New Zealand Hauraki Gulf region, but the apparent trend towards expansion of algal bloom distribution suggest that BTX-group toxins are emerging in other regions in the world. To date BTX-group toxins have not been reported in shellfish or fish from Europe (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2010b).

Signs and symptoms of palytoxin (PlTX)-group toxins intoxication are not well-defined, but include myalgia and weakness, possibly accompanied by fever, nausea and vomiting. PlTX-group toxins have mainly been detected in marine zoanthids (soft corals) of the genus *Palythoa* and in benthic dinoflagellates of the genus *Ostreopsis* (e.g. *Ostreopsis siamensis*, *O. mascarenensis*, *O. ovata*). Blooms of *Ostreopsis* have been reported in European countries such as France, Greece, Italy and Spain (EFSA, 2009e).

3.4. Summary of conclusions on hazard identification and characterisation

3.4.1. Microbiological hazards

Based on incidents of food and waterborne infection, the properties and the distribution of the agents, microbiological hazards (include viruses, bacteria and parasites) have been identified as associated with seawater. Poor quality sea water may consequently have a severe impact on public health through contamination which may occur during food processes. The hazards are associated either with bacteria which are part of the natural marine biota (*Vibrio* spp.), or pathogenic microbes derived from animal or human faecal contamination (norovirus, hepatitis A virus, *Salmonella*) which is most often of terrestrial origin. However, there is currently not sufficient data to estimate the public health risks associated with the uses in on-land establishments for handling and washing fishery products, for the production of ice used for chilling and for rapid cooling of crustaceans and molluscs after cooking. The same hazards may occur in seawater used for bottling although there is a lack of observational data on this product.

The above mentioned microbiological hazards, if present in seawater, are also likely to be present on the surfaces of fresh fishery product, but will be concentrated from seawater by filter-feeding shellfish and fish prior to harvest. Following preparation of fishery products (gutting, heading, slicing, filleting, and chopping) some bacterial hazards (e.g. *Vibrio* and *Clostridium botulinum*) will increase as a result of their growth if permissive storage conditions are provided. Consequently, the public health risks associated with the use of seawater for unprocessed product are likely to be less than those where seawater is used for operations where the seawater comes in contact with processed food or ready-to-eat food (water and ice used for handling and washing of prepared fishery products, water and ice used for rapid cooling of crustaceans and molluscs after their cooking). The same consideration applies to bottled seawater as this may be consumed as a ready-to-eat product.

3.4.2. Chemical hazards

Both inorganic and organic chemicals can be found in seawater in concentrations that are usually low. Therefore, the use of seawater on fresh or processed fishery products or for the revitalisation of molluscs is unlikely to raise a health concern. A potential health concern may occur from the use of bottled seawater where human exposure might be expected to be higher than for the other uses of seawater, indicating that more rigid criteria are needed for bottled seawater (see also Sections 4.7.1 and 4.7.2).

3.4.3. Algae

Toxic algae species could have a serious impact on human health. Outbreaks of toxic syndromes are primarily related to consumption of filter-feeding bivalve molluscs, that had concentrated the toxins present in the algae in their digestive gland (hepatopancreas), rather than to direct contact with hazardous algae species in seawater.

4. Hygiene criteria for clean seawater

The WHO Guidelines for drinking-water quality (WHO, 2011[16]) states that "the framework for safe drinking-water is a preventive management approach [comprises] three key components:

[16] www.who.int/water_sanitation_health/publications/2011/dwq_chapters/en/index.html

1. health-based targets based on an evaluation of health risks

2. water safety plans (WSPs), comprising:

   - a system assessment to determine whether the drinking-water supply (from source through treatment to the point of consumption) as a whole can deliver water of a quality that meets the health-based targets;

   - operational monitoring of the control measures in the drinking-water supply that are of particular importance in securing drinking-water safety;

   - management plans documenting the system assessment and monitoring plans and describing actions to be taken in normal operation and incident conditions, including upgrade and improvement, documentation and communication;

3. a system of independent surveillance that verifies that the above are operating properly.

A similar process to that outlined by the WHO for drinking water supply is used here for seawater quality and the practices of assessment and water treatment are standard in the potable water supply industry. The use of sanitary surveys (surveys of the faecal pollution inputs, and their potential circulation within a given marine environment) for the control of faecal pollution in shellfish growing water has recently been reviewed (EFSA Panel on Biological Hazards (BIOHAZ), 2011) and this process is equivalent to that of assessment to determine the quality of source water used in drinking-water supply as part of the WSP outlined in the WHO Guidelines for drinking-water quality (WHO, 2011). The use of sanitary surveys is outlined and further discussed in Section 4.4.1. Apart from the requirement for removal of surface contamination, EC Regulation 853/2004[9] requires food business operators in other areas of food production to use water of potable water quality. A similar approach to hygiene criteria based on the risk associated to the intended use of seawater is proposed here. Different criteria are therefore proposed according to the intended uses of seawater, considering that the contamination risks are not the same. When seawater is used for operations where the seawater comes in contact with processed food or ready-to-eat food (water and ice used for handling and washing of prepared fishery products, water and ice used for rapid cooling of crustaceans and molluscs after their cooking), it is considered that there is a risk of microbial contamination of the product and the public health risks are greater. The same consideration has been taken for bottled seawater, as this product may be ingested directly without any further treatment or culinary preparation. This provides consistency with the EC Regulation 853/2004 requiring food business operators to use water of potable water quality and is also consistent with the recommendations of the Codex Guidelines for *Vibrio* in Seafood (CAC/GL 73-2010).

4.1. Health-based targets

As already outlined, poor quality sea water may have a severe impact on public health although there is currently not sufficient data to estimate the public health risks associated with the uses in on-land establishments for handling and washing fishery products, for the production of ice used for chilling and for rapid cooling of crustaceans and molluscs after cooking as well as for bottled seawater used for domestic use. In the absence of data to propose risk-based criteria, hazard-based criteria are proposed instead. These should provide the same level of health protection as achieved by other food business operators through the use of potable water.

4.2. Microbiological criteria - Water safety plans

4.2.1. Water Supply

Water of potable quality either requires identification of a pristine source (such as for natural mineral water where all possible precautions are taken within the protected perimeters to avoid any pollution of, or external influence on, the chemical and physical qualities), or requires specific treatments

capable of removing the range of contaminants (pathogenic organisms and chemicals) of public health concern. Coastal sources, used for abstraction of seawater in land-based establishments, cannot be guaranteed to be free from pathogens from the marine biota or from faecal contamination, and cannot be classified as a pristine source.

The use of sanitary surveys for the control of faecal pollution in shellfish growing water has recently been reviewed (EFSA Panel on Biological Hazards (BIOHAZ), 2011) and this process is equivalent to that of assessment to determine the quality of source water used in drinking-water supply as part of the WSP outlined in the WHO Guidelines for drinking-water quality (WHO, 2011). Faecal indicator legislative standards govern shellfish production in the EU and in third countries importing into the EU. Competent Authorities in EU Member States are required to define the location and boundaries of production (and relaying) areas and to classify the areas according to one of the three categories. They are further required to establish a sampling (monitoring) programme, which should be representative, to ensure that bivalve molluscs harvested from the area comply with the established classification. If bivalves do not comply with the criteria the Competent Authority must close or reclassify the area. An essential first step prior to setting up a sampling programme is to perform a sanitary survey within the production area, so that sampling points can be determined as representative according to scientific principles. This sanitary survey is a requirement of both US[17] and EU regulations.[18] However, in the EU this only applies to areas classified after 2006 and hence monitoring programmes for the majority of production areas in the EU (which were established prior to 2006) are not based on sanitary surveys. EU legislation does not contain detailed rules for implementation of monitoring programmes – for example key aspects, such as the required monitoring frequency, are not specified. An EU working group has drawn up detailed best practice guidance; however compliance with these rules is not currently mandatory. For example, future legislation may require a mandatory harvesting prohibition zone round all human discharge sources (a minimum distance or dilution criteria could be established). Such measures are already incorporated into bivalve mollusc sanitation legislation in countries outside of the EU. However, this could only be applied if a sanitary survey had been performed in the production area, and thus, the pollution sources were documented. This measure would thus also require sanitary surveys to be performed for all production areas (see above) as a first step. Sanitary surveys also provide the fundamental pollution impact data needed to consider proactive management of bivalve production areas.

Although sanitary surveys will provide information on optimal abstraction sites to control sources of faecal pollution, additional considerations will be needed to reduce contamination from endogenous marine flora (including pathogenic *Vibrio* spp. and *C. botulinum*). Since these hazards are associated with temperature and salinity (*Vibrio* spp.) as well as sediments (*C. botulinum)*, abstracting seawater with high salinity and free from particulate material (especially in waters of temperatures below 20 ºC, will increase the seawater quality prior to treatment.

Options to control hazards in clean seawater include a combination of sanitary surveys, with either microbiological standards, and appropriate water treatment will be necessary (for detailed description of treatment methods see Annex B). Treatment options should be based on the multiple-barrier principle. The strength of this approach is that a failure of one barrier may be compensated by effective operation of the remaining barriers, thus minimizing the likelihood of hazards passing through the entire system and being present in the final treated water. Treatment options include pre-treatment, filtration (sometimes combines with other processes) and disinfection. Treatment options will have to be designed on a case-by-case basis and consider both the hazards from faecal pollution as well as these from the endogenous marine flora (including pathogenic *Vibrio* spp. and *C. botulinum*).

[17] www.fda.gov/Food/FoodSafety/Product-SpecificInformation/Seafood/FederalStatePrograms/NationalShellfishSanitationProgram/ucm046353.htm

[18] Directive 2006/113/EC of the European Parliament and of the Council of 12 December 2006. OJ L 376, 27.12.2006, p. 14–20. 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. OJ L 64, 4.3.2006, p. 37-51.

Control of these hazards will need a validation stage to show that the treatment processes can achieve required reduction levels of a wide range of hazards (see Section 3). This validation will have to be established on a case-by-case basis for the various applications considered here. Validation should be undertaken during pilot stage studies or during initial implementation of water treatment system. It should provide assurance of adequate reduction of hazards from faecal pollution as well as those from the endogenous marine flora but is not used for day-to-day operational monitoring.

4.2.2. Operational monitoring

The WHO (WHO, 2011) advice on water quality is equally applicable to seawater used in land-based food production environments as outlined above. This advice concluded; "Owing to issues relating to complexity, sensitivity of detection, cost and timeliness of obtaining results, testing for specific pathogens is generally limited to assessing raw water quality as a basis for identifying performance targets and validation, where monitoring is used to determine whether a treatment or other process is effective in removing target organisms". Furthermore, the WHO concluded that "Drinking-water safety is secured by application of a WSP, which includes monitoring the efficiency of control measures using appropriately selected determinants. In addition to this operational monitoring, a final verification of quality is required. Verification is the use of methods, procedures or tests in addition to those used in operational monitoring to determine whether the performance of the drinking-water supply is in compliance with the stated objectives outlined by the health-based targets and whether the WSP needs modification or revalidation." Operational monitoring of the control measures applied to sea water will be dependent on the treatment applied, and microbial parameters are likely to be inappropriate for this purpose. Treatment parameters may include disinfectant concentration and contact time, ultraviolet intensity, pH, light absorbency, membrane integrity, turbidity and colour etc. However, for validation, which is not used for day-to-day management of the water supplies, parameters can be chosen to reflect the microorganisms being targeted by treatment. Therefore, dependent on the treatment applied to seawater, parameters for hygiene criteria, based on microbiological indicators used for drinking water, may provide a similar level of health protection. If however the sanitary surveys are effective in excluding faecal contaminants, detection of additional indicators based on the marine microbiota may be necessary for hygiene criteria since *E. coli* and Enterococci will not be present in the water prior to treatment. It is therefore proposed here to include an additional hygiene criteria based on the detection of all *Vibrio* species since these are more likely to be present in all seawaters, and may act as an indicator for the removal of pathogenic *Vibrio* spp. and thus provide a target for the water treatment.

4.2.3. Management plans

Management plans will need to be established which describe actions to be taken to maintain optimal operation under normal operating conditions. These will include both responses to normal variations in operational monitoring parameters and responses when operational monitoring parameters reach critical limits. All activities, including standard operating procedures applied during normal conditions and planned responses to incidents and emergencies, should be documented. The critical limits will need to be established on a case-by-case basis as a result of validation and pilot stage studies and as above are standard practices in the water supply industry.

4.2.4. Independent surveillance for verification

Although surveillance will not need to be as extensive as for drinking water supply, there will still be a need for public health oversight and processes for approval of water safety plans (WSPs). This approval will normally involve review of the system assessment, of the identification of appropriate control measures and supporting programmes and of operational monitoring and management plans.

It should ensure that the WSP covers normal operating conditions and predictable incidents (deviations) and has contingency plans in case of an emergency or unplanned events. The surveillance and verification process will have common features with that governing shellfish production.

4.3. Parameters to verify efficacy of treatment

There exist various microbiological criteria affecting waters which are already in force in the European legislation and elsewhere (see Appendix A). These are based on indicator organisms. Indicator organisms can provide a measure of microbiological quality including contamination from the environment and a measure of faecal contamination. The most commonly used bacteriological indicator organisms used are coliforms, *E. coli*, enterococci, and *Clostridium perfringens* or sulphite-reducing clostridia.

4.3.1. *Escherichia coli*

This species has a precise taxonomic definition, is easily identifiable, and has good specificity for faecal contamination since the bacterium survives for brief periods in the environment. Consequently, their presence as an indicator organism for faecal contamination of environmental samples is frequently used for routine analyses. *E. coli* is particularly important in the context of existing microbiological criteria for live bivalve molluscs, echinoderms, tunicates and gastropods (Reg. (EC) No 2073/2005[19]), as well as bathing waters (Directive 2006/7/EC[20]).

4.3.2. Enterococci

The genus *Enterococcus* comprises facultative anaerobic cocci able to survive in a wide range of environmental conditions, growth at temperatures between 10 and 45 °C, resistant to 60 °C for 30 minutes, growth at pH 9.6 and at 6.5 % NaCl, and with ability to hydrolyze esculin in presence of 40 % bile. Previously classified as Group D *Streptococcus*, they have been also named faecal streptococci and intestinal enterococci (Bartram and Rees, 2000).

Enterococci are typically excreted in the faeces of humans and other warm-blooded animals and are present in large numbers in sewage and water environments polluted by sewage or wastes from humans and animals, therefore this group is used as an index of faecal pollution. The numbers of enterococci in human faeces are generally about an order of magnitude lower than those of *E. coli*. However, they tend to survive longer than *E. coli* in water environments, namely in marine waters (Borrego et al., 1983; Evison and Tosti, 1980) and persistence patterns are similar to those of water-borne pathogenic bacteria (Sinton et al., 1993). Enterococci are particularly important in the context of existing microbiological criteria for bathing waters.

4.4. Proposed microbiological criteria for use in on-land establishments

4.4.1. Sanitary survey and microbiological criteria to be applied to clean seawater and ice intended for handling, washing and chilling of whole fishery products

When seawater is used for purposes that do not involve a direct contact with food (physical cleaning operations of utilities, surfaces, floors, equipment in food facilities such as fish markets, auctions, fishery ports) or for the cleaning of whole raw fishery products the exposures associated with those operations are low.

Hygiene criteria based on public health protection currently exist for bathing water. Schets and colleagues (2011) estimated exposure for swallowing water through recreational bathing. This study estimated that men swallowed on average 27-34 ml per swimming event, women 18-23 ml, and children 31-51 ml and varied dependent on the water type. Since there is not sufficient data to estimated the public health risks associated with the uses of seawater in on-land establishments and assuming that contamination of raw fishery products and the use of clean sea water used for removal of surface contamination during processing involves a similar or lower exposure to recreational bathing, application of standards used for bathing water to clean seawater is likely to provide a similar level of public health protection.

[19] OJ L 338, 22.12.2005, p. 1–26.
[20] OJ L 64, 4.3.2006, p. 37–51.

The hygienic quality of seawater and ice can be assessed by combining a sanitary survey procedure with the assessment of microbial source water quality. The same approach is applied to ice derived from clean seawater.

Information on potential sources of pollution in the vicinity of the intended abstraction source should be determined. This constitutes the sanitary survey. Examples of elements of a sanitary survey (CEFAS, 2010) that could be considered will include identification of information on:

Desk study

- Human population centres
- Sewage discharges and septic tanks
- Storm-water discharges
- Locations of major watercourses (rivers, large streams)
- Locations of ports and marinas

On the basis of information collected from the sanitary survey the operator should consider;

- Whether the area is appropriate for abstraction
- If so, the location of the abstraction point to minimise risk
- The timing of abstraction to minimise risk, if relevant

As outlined in Section 4.2.1, the abstraction point survey should also consider temperature, salinity and particulate material to reduce contamination by microbiological hazards which are part of the marine biota. Pathogenic species of *Vibrio* show ecological preference for estuarine areas of moderated salinity and their occurrence declines when salinity increases above 30 ppt. These species may grow at temperatures as low as 13 °C but their concentration in the environment is low at temperatures below 16 °C with a highest abundance when the water temperatures are above 20 °C. Recent studies suggest turbidity may also affect *V. parahaemolyticus* levels in shellfish, although the extent of its influence is still uncertain.

It is recommended to use the following criteria equivalent to those in Directive 2006/7/EC on bathing water quality (excellent quality), which will be in force starting from 2014, as a standard for source waters rather than for process verification. If unable to meet these microbiological criteria, the seawater should not be used.

Table 2: Proposed criteria to be applied to clean seawater intended for handling, washing and chilling of whole fishery products

Parameter	Parametric value	Reference method of analysis
Escherichia coli	250/100 ml*	ISO 9308-3 or ISO 9308-1
Enterococci	100/100 ml*	ISO 7899-1 or ISO 7899-2

*Based upon a 95-percentile evaluation

The approach taken in the Bathing Waters Directive to calculate the 95-percentile is based fitting a lognormal probability density function to the observed data. The advantage of this method is that a 95-percentile can be estimated even if only a limited number of samples has been analysed. However, the result is only valid if the assumption of a lognormal distribution is reasonably well satisfied, and if the mean and standard deviation can be estimated without bias. This may be problematic if zero values are included in the dataset. According to the Bathing Waters Directive, these are replaced by the detection limit of the analytical method. Recent work has suggested that this may result in biased estimates of the mean standard deviation and alternative methods for analysis of such censored data based on maximum likelihood estimation or Bayesian statistics have been proposed (Busschaert et al., 2010;

EFSA, 2011). Non-parametric approaches may also be considered (Bartram and Rees, 2000; Ellis, 1989; Lorimer and Kiermeier, 2007). Further guidance should be provided to food business operators how to evaluate data from microbiological analysis of sea water.

4.4.2. Sanitary survey and criteria to be applied to clean seawater and ice intended for handling, washing and chilling of prepared and/or processed fishery products, and for rapid cooling of crustaceans and molluscs after their cooking

Higher exposure to microbiological hazards will occur where seawater will be in contact with prepared, processed, and/or ready-to-eat fishery products. For these uses it is recommended that the hygienic quality of seawater is assured by source water protection combined with actions resulting from a more comprehensive sanitary survey and mandatory water treatment (see Appendix B). The same requirement for sanitary surveys and treatment applies to clean seawater used for the production of ice.

Elements of a comprehensive sanitary survey that could be considered, in addition to those presented in 4.4.1., will include identification of information on:

Desk study

- Locations and timing of sewage sludge spreading
- Locations of farms, slurry storage tanks and any permitted discharges
- Locations of major wildlife populations (e.g. deer herds and grazing sites, wildfowl/seabird breeding sites)
- Locations of all watercourses (rivers, streams)
- Locations of ports, marinas and moorings ; determine whether there are any pump-out facilities and/or controls on discharges from boats
- Tidal characteristics and current flows

Shoreline survey

- Undertake a shoreline survey of the area in the vicinity of the proposed abstraction point in order to determine whether there are any sources that have not been identified through the desk study. Also look for sewage-related debris.

Laboratory analysis

- Testing of samples from the proposed abstraction site for turbidity and faecal indicator organisms over a range of tidal and weather conditions.

On the basis of information collected from the sanitary survey the operator should consider;

- Whether the area is appropriate for abstraction
- If so, the location of the abstraction point to minimise risk
- The timing of abstraction to minimise risk, if relevant
- the appropriate treatment method(s)

As outlined in Section 4.4.1, the abstraction point survey should also consider seawater temperature, salinity and particulate material to reduce contamination from microbiological hazards in the marine biota.

It is recommended to use the following criteria equivalent to those in the drinking water regulation (Council Directive 98/83/EC) process and an additional criterion for *Vibrio* spp. as a tool for monitoring the efficacy of the treatment.

Table 3: Proposed criteria to be applied to clean seawater intended for handling, washing and chilling of prepared and/or processed fishery products, and for rapid cooling of crustaceans and molluscs after their cooking

Parameter	Parametric value	Reference method of analysis
Escherichia coli	0/100 ml	ISO 9308-1
Enterococci	0/100 ml	ISO 7899-2
Vibrio spp.	0/100 ml	ISO/TS 21872-1:2007 or ISO/TS 21872-2:2007

4.5. Proposed microbiological criteria to be applied to bottled seawater for domestic uses

The same considerations as in 4.4.2. apply for the need to apply water treatment and to undertake sanitary surveys.

In addition, more stringent criteria for bottled seawater are recommended, because of the prolonged shelf life, the intended use in ready-to-eat products (e.g. salad dressings), and the potential concentration of hazards by bivalve molluscs during revitalisation. It is recommended to use the following criteria equivalent to those in the drinking water regulation (Council Directive 98/83/EC[21]; water offered for sale in bottles or containers) as a tool for monitoring the efficacy of the treatment process. As discussed above, seawater cannot be considered as a pristine source, and treatment will be necessary to assure that the microbiological criteria will be met. As in the drinking water regulation, a turbidity standard of lower than 1 NTU is proposed to assure that water treatment effectively removes particulate matter, while also preventing interference with disinfection. As the concentrations of faecal indicator bacteria may be low in source waters, they cannot be used to assess treatment effectiveness against indigenous species. Therefore, an additional criterion for total *Vibrio* spp. is proposed as an indicator of efficient removal of pathogenic vibrios.

Table 4: Proposed criteria to be applied to bottled seawater for domestic uses

Parameter	Parametric value	Reference method of analysis
Turbidity	< 1 NTU	ISO 7027*
Escherichia coli	0/250 ml	ISO 9308-1
Enterococci	0/250 ml	ISO 7899-2
Vibrio spp.	0/250 ml	ISO/TS 21872-1:2007 or ISO/TS 21872-2:2007

*Four methods are given in that standard and the appropriate one to use for this application is the quantitative method using measurement of diffuse radiation.

4.6. Detection methods to verify compliance with microbiological criteria

Methods for *E. coli* and enterococci are defined in the international standards (ISO 9308-3 or ISO 9308-1 for *E. coli* and ISO 7899-1 or ISO 7899-2 for enterococci). Risk of *Vibrio* illness has been related to seafood consumption and consequently surveillance systems have been orientated toward the detection of *Vibrio* in food products. Therefore, standard methods for the investigation of total vibrios in water are not currently available and the existing reference methods for the detection of *Vibrio* in seafood (ISO/TS 21872-1:2007 or ISO/TS 21872-2:2007) need to be adapted for their application to seawater analysis. Alternatively, a membrane filtration on a selective medium for *Vibrio* on the basis of other ISO standard methods for the examination of water can be applied for measuring the total number of *Vibrio* in seawater. Outline descriptions of these methods are listed in Appendix C.

[21] OJ L 330, 5.12.1998

4.7. Proposed chemical criteria to be applied to clean seawater

As indicated in Chapter 2, clean seawater may be used for: a) cleaning of facilities and equipment, b) manufacture of ice for cooling and storage of fishery products, either fresh or processed, c) washing of whole, gutted, and beheaded fishery products and handling and washing of unprocessed products such as fish fillets and slices, d) cooling of crustaceans and molluscs after cooking, and e) to be bottled for domestic food preparation activities.

4.7.1. Clean seawater intended for handling, washing and chilling of whole or prepared fishery products, and for rapid cooling of crustaceans and molluscs after their cooking

Considering the usually low levels of chemical contaminants in seawater the use of clean seawater as indicated under a) to d) can be considered not to contribute in a significant manner to exposure of consumers and could therefore be considered not to pose a health concern. Nevertheless this seawater should be clean and information on local point sources of pollution in the vicinity of the intended abstraction source should be determined, such as:

- Possible municipal pollution
- Proximity of industrial activities or nuclear power plants
- Proximity of agricultural activities
- Possible local oil spills or oil discharges from boats
- Location of river estuaries

Based on the information collected the operator should conclude on the appropriateness of the seawater abstraction point.

4.7.2. Bottled seawater for domestic use

The use of bottled seawater for home cooking of fish, lobster or pasta, for bread baking, pizza dough or savoury pastry, as a component of salad dressing by mixing with either oil or vinegar for salad dressing and for re-vitalisation of live bivalve molluscs, may pose a potential health risk for the consumer.

The same considerations on the suitability of the abstraction source of the seawater as in Section 4.7.1 apply. In addition, consideration should be given to abstraction deep in the water column (off-shore) as the preferred source of seawater for the production of bottled seawater.

In addition, to these considerations, stringent criteria for bottled seawater should be applied. In line with the requirements for food business operators to use water of potable water quality laid down in Regulation 853/2004[9] it is concluded that the same approach should be applied for bottled seawater which will be placed on the market. Standards for chemicals (parameter values) in potable water have been laid down in Council Directive 98/83/EC[12] on the quality of water intended for human consumption, which must be met by drinking water within the European Union and establishes strict quality standards for water used for human consumption. In this directive 'water intended for human consumption' is defined as all water either in its original state or after treatment, intended for drinking, cooking, food preparation or other domestic purposes, regardless of its origin and whether it is supplied from a distribution network, from a tanker, or in bottles or containers. In addition, Commission Directive 2003/04/EC[13] establishes the list, concentration limits and labelling requirements for the constituents of natural mineral waters. Both directives aim at protection of the consumer against unwanted effects of hazardous chemicals in water. Therefore safe bottled seawater should fulfil the criteria for chemical parameters as laid down in Directive 98/83/EC[12] (see Table 5 Appendix A). Additionally, for two other chemical contaminants, barium and manganese, maximum limits have been set in Annex 1 of Commission Directive 2003/04/EC[13] (see Table 6 Appendix A).

It should, however, be noted that Council Directive 98/83/EC[12] covers drinking water. For the risk assessment of chemicals in drinking water usually a consumption of two litres per day by a 60 kg adult is considered. Although no data on the consumption of bottled seawater are available, it can be assumed that this might be much less since it is not intended for drinking, but for cooking, salad dressing and re-vitalisation of live molluscs. Therefore, applying the criteria laid down in Council Directive 98/83/EC[12] will provide a high level of protection for consumers using bottled seawater.

The levels in seawater presented in Table 1 (Chapter 3.2.3) for the different chemicals are low and all below the respective parameter values laid down in the Council Directive, and presented in Table 5 of Appendix A, with one exception. The mean occurrence value for boron is 3.6 mg/l (range 0.7 - 4.9 mg/l), meaning that this value is well above its parameter value of 1 mg/l in Council Directive 98/83/EC[12] and also above the WHO guideline value of 2.4 mg/l.

Considering reported high levels of boron (up to 4.3 mg/l) in natural mineral waters and the upper levels (ULs) established by the NDA Panel (EFSA, 2004), the CONTAM Panel concluded that it is very unlikely that adults and children older than 14 years would exceed these ULs even at the highest reported levels in bottled natural mineral water. For children from 1 to 14 years of age, a maximum limit of 1.5 mg boron/l in bottled natural mineral water would protect these children from exceeding the UL. However this latter conclusion was based on the same water consumption estimate as for adults. When a more realistic scenario was used, a maximum concentration of 4.3 mg/l would be unlikely to lead to exceedance of even the lowest UL value (EFSA, 2005d). It should be noted, however, that other sources (i.e. diet) of exposure to boron were not taken into account in this assessment.

Regarding the use of bottled seawater it can be assumed that consumption would be less than that of bottled mineral water. However, no data are available on the consumption of bottled seawater. Therefore, in the case of boron, operators should measure boron levels in seawater and make an assessment of whether these levels might pose a risk for human health considering the consumption of bottled seawater. In that case specific treatment with a selective boron ion exchange resin should be considered to bring the boron concentration below its parameter value of 1 mg/l.

Reported concentrations of fluoride in seawater (1.2 – 1.5 mg/l) were in the range of the parameter value of 1.5 mg/l in the Council Directive. Although no data on the consumption of bottled seawater are available yet, it can be assumed that consumption will be less than for drinking water. Therefore, it is unlikely that bottled seawater containing fluoride at concentrations below the parameter value stated in Council Directive 98/83/EC[12] would pose a health concern.

In Chapter 3.2.3 (Table 1) it was shown that for acrylamide, epichlorohydrin and vinyl chloride no occurrence data are available. Since these compounds are particularly used in drinking water treatment and transport, it can be expected that the concentration in seawater will be low. It is therefore recommended that operators determine the levels of these chemicals in seawater to investigate whether continuous monitoring is needed.

Bromate and trihalomethanes are disinfection by-products that are formed during the use of disinfection processes, respectively ozonation or chlorination. In seawater the presence of organic matter could contribute to the formation of these hazardous disinfection by-products. But, in particular the high concentration of bromide, could be an important contributor. Mean concentrations of bromide in seawater have been reported to range from 65 to 70 mg/l, whereas concentrations in fresh water are usually lower than 0.2 mg/l (Flury and Papritz, 1993). When UV or other physical methods such as filtration are used as disinfection method these disinfection by-products will not be formed and operators should consider whether these methods could be used as the preferred disinfection process in order to prevent formation of bromate and trihalomethanes.

4.8. Proposed criteria to be applied to clean seawater related to phytoplankton/algae

The presence of toxic algae in source water, particularly in coastal water may pose a potential health risk for the consumer. However, due to their size algae can effectively be removed by sand or (micro)filtration (see Annex B). Dinoflagellates such as *Alexandrium spp.*, *Ostreopsis spp.* and *Karena brevis* are nearly spherical and have a diameter ranging from about 20-70 μM. *Chondria spp.* have a different shape and could reach a size of several millimetres.

Although the presence of toxic algae in clean seawater can effectively be prevented by filtration, it is however possible that a certain number of toxic algae cells or toxins, if the cells are disrupted, can settle on whole or freshly prepared fishery products. It should be noted, however, that it is expected that in that case levels of marine toxins will be much lower than those reached by bio-accumulation of toxins in bivalve molluscs or fish.

The amount of toxin-producing algae cells can vary considerably over the year. Periods of explosive growth ('hazardous algae bloom') can occur during changes in weather conditions, but other factors such as upwelling, temperature, turbidity, turbulence or salinity of the water may also play a role. Human activities can increase nutrient inputs through changes in land-use patterns or changes in the hydrology of an area, facilitating occurrence of hazardous algae bloom (FAO, 2004). In addition, also hydrographical conditions such as the presence of a thermocline, an upper layer of seawater which does not mix with the underlying water layers, is an important factor for algae growth. Therefore, the operator should monitor the conditions that affect blooms of toxic algae with the aim of preventing human exposure.

Therefore local conditions such as:

- Municipal pollution
- Euthrophication
- Turbidity
- Temperature
- Algae growth/proliferation

should be monitored.

In addition, consideration should be given to the conditions for abstraction of the source water:

- During high tide
- Not during periods of dredging or storms
- Deep in the water column, preferentially offshore.

4.8.1. Methods to monitor hazardous algae bloom

There are no prescribed, official, methods or programmes to monitor hazardous algae bloom. Historically, detection of algae has relied on microscopic methods for distinguishing morphological characters, but this is a rather laborious task. Nowadays, the development of molecular probes is enabling detection of lower concentrations of cells, and provides the potential to allow for discrimination of unique algae species. Remote sensing techniques, including satellite and airplane over flights, as well as *in situ* devices, hold great promise for improvement of monitoring hazardous algae bloom (National Sea Grant College Program, 2001; Hallegraeff et al., 2003).

4.9. Detection methods to verify compliance with chemical criteria

Performance characteristics for the method of analysis for the chemical parameters included in Directive 98/83/EC[12] have specified in Annex III, article 2 of this directive.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- Based on incidents of food and waterborne infection, the properties and the distribution of the agents, microbiological hazards (including viruses, bacteria and parasites) have been identified as associated with seawater. Poor quality sea water may consequently have a severe impact on public health through contamination which may occur during food processes. The hazards are associated either with bacteria which are part of the natural marine biota (in particular *Vibrio* spp.), or pathogenic microbes derived from animal or human faecal contamination, which is most often of terrestrial origin.

- There is currently not sufficient data on microbiological hazards to estimate the public health risks associated with the uses in on-land establishments for handling and washing fishery products, for the production of ice used for chilling, for rapid cooling of crustaceans and molluscs after cooking, and for bottled seawater.

- In the absence of data to propose risk-based criteria, hazard-based criteria are proposed instead, These should provide the same level of health protection as achieved by other food business operators through the use of potable water.

- Coastal sources, used for abstraction of seawater in land-based establishments, cannot be guaranteed to be free from pathogens from the marine biota or from faecal contamination, and cannot be classified as a pristine source.

- Sanitary surveys provide information to optimize the site of abstraction in order to control sources of faecal pollution and chemical contamination. Additional safeguards will be needed to reduce contamination from endogenous marine flora (including pathogenic *Vibrio* spp. and *C. botulinum*). Since these hazards are associated with temperature and salinity (*Vibrio* spp.) as well as sediments (*C. botulinum)*, abstracting seawater with high salinity (especially in waters of temperatures below 20 ºC), and free from particulate material will improve safety of seawater prior to treatment.

- The comprehensiveness of the sanitary survey, the stringency of microbiological criteria and the need for treatment will depend on the relative exposures associated to the different uses of clean seawater;

    - When seawater is used for purposes that do not involve a direct contact with food (physical cleaning operations of utilities, surfaces, floors, equipment in facilities such as fish markets, auctions, fishery ports) or do not convey a contamination risk with prepared fishery products (e.g. handling and washing whole fishery products), it is considered that the exposure will be low. For these uses, a basic sanitary survey and microbiological criteria based on the Directive 2006/7/EC are considered appropriate.

    - Higher exposure to microbiological hazards will occur where seawater will be in contact with prepared, processed, and/or ready-to-eat fishery products. For these uses, a more comprehensive sanitary survey, mandatory water treatment and microbiological criteria based on Council Directive 98/83/EC and an additional criterion for *Vibrio* spp. are considered appropriate.

    - Highest exposure to microbiological hazards occurs where seawater is used for revitalisation of live bivalve molluscs, as a component of salad dressings or other similar uses as ingredient of ready-to-eat products. For these uses, a more comprehensive sanitary survey, mandatory water treatment and microbiological criteria based on Council Directive 98/83/EC for water offered for sale in bottles and an additional criterion for turbidity and *Vibrio* spp. are considered appropriate.

- For verification of treatments, detection methods for *E. coli* and enterococci are defined in the international standards (ISO 9308-3 or ISO 9308-1 for *E. coli* and ISO 7899-1 or ISO 7899-2 for enterococci). Reference methods for the detection of *Vibrio* in seawater have not been evaluated.

- The concentration of chemicals in bottled seawater should comply with the maximum levels for chemical contaminants (parameter values) as laid down in Council Directive 98/83/EC on the quality of water intended for human consumption.

- Concentrations of boron in seawater are well above the parameter value of 1 mg/l. Therefore operators should measure boron levels in seawater and make an assessment of whether these levels might pose a risk to human health, given the consumption of bottled seawater, and consider whether treatment with a selective boron ion exchange resin is needed to bring the boron concentration below its parameter value.

- Appropriate treatment (filtration) is needed to prevent a potential health risk from the presence of toxic algae in source water.

RECOMMENDATIONS

- Reference methods for the detection of *Vibrio* in seafood (ISO/TS 21872-1:2007 or ISO/TS 21872-2:2007) should be applied to seawater with appropriate modification. Alternatively, a membrane filtration on a selective medium for *Vibrio* on the basis of other ISO standard methods for the examination of water can be applied for measuring the total number of *Vibrio* in seawater. These should be evaluated involving multi-laboratory comparison, the use of external quality assessment and appropriate reference materials.

- Further guidance and documentation should be made available for sanitary survey and percentile calculations for managers of on-land establishments.

- Data on the consumption of clean seawater are needed to facilitate further risk assessments.

- Operators should determine the levels of acrylamide, epichlorohydrin and vinyl chloride in seawater to investigate whether continuous monitoring is needed.

- Since ultraviolet or other physical methods do not result in the production of hazardous disinfection by-products such as bromate and trihalomethanes, it is recommended to use these methods as the preferred disinfection process.

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APPENDICES

A. CURRENT EU LEGISLATION

Parameter	Guide	Mandatory	Method	Legislation
Faecal coliforms/100 ml	≤ 300 in the shellfish flesh and intervalvular liquid		Method of dilution with fermentation in liquid substrates in at least three tubes in three dilutions. Subculturing of the positive tubes on a confirmation medium, Count according to MPN. Incubation temperature 44˚C ± 0.5˚C	Directive 2006/113/EC on the quality required for shellfish waters
Total coliforms/100ml	500	10,000	Fermentation in multiple tubes. Subculturing of positive tubes on a confirmation medium. Count according to MPN or membrane filtration and culture on an appropriate medium such as Tergitol lactose agar, endo agar, 0.4 % Teepol broth, subculturing and identification of the suspect colonies.	Council Directive of 8 December 1975 concerning the quality of bathing water (76/160/EEC)
Faecal coliforms/100 ml	100	2,000	Fermentation in multiple tubes. Subculturing of positive tubes on a confirmation medium. Count according to MPN or membrane filtration and culture on an appropriate medium such as Tergitol lactose agar, endo agar, 0.4 % Teepol broth, subculturing and identification of the suspect colonies.	Council Directive of 8 December 1975 concerning the quality of bathing water (76/160/EEC)
Faecal streptococci/ 100 ml	100	-	Litsky method. Count according to MPN or filtration on membrane. Culture on an appropriate medium. Fermentation in multiple tubes. Subculturing of positive tubes on a confirmation medium. Count according to MPN or membrane filtration and culture on an appropriate medium such as Tergitol lactose agar, endo agar, 0.4 % Teepol broth, subculturing and identification of the suspect colonies.	Council Directive of 8 December 1975 concerning the quality of bathing water (76/160/EEC)
Salmonella/1 l	-	0	Concentration by membrane filtration. Inoculation on a standard medium. Enrishment – subculturing on isolating agar - identification	Council Directive of 8 December 1975 concerning the quality of bathing water (76/160/EEC)
Enteric viruses PFU/10 l	-	0	Concentration by filtration, flocculation or centrifuging and confirmation.	Council Directive of 8 December 1975 concerning the quality of bathing water (76/160/EEC)
Escherichia coli/250 ml		0		Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption

Parameter	Guide	Mandatory	Method	Legislation
Enterococci/250 ml		0		Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption
Pseudomonas aeruginosa/250 ml		0		Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption
Colony count 22˚C		100		Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption
Colony count 37˚C		20		Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption
Escherichia coli and other coliforms/250 ml		0	Rivivable colony count. Incubation temperature 37˚C and 44.5˚C	Directive 2009/54/EC on the exploitation and marketing of natural mineral waters
Faecal streptococci/250 ml		0		Directive 2009/54/EC on the exploitation and marketing of natural mineral waters
Sporulated sulphite-reducing anaerobes/ 50 ml		0		Directive 2009/54/EC on the exploitation and marketing of natural mineral waters
Pseudomonas aeruginosa/250 ml		0		Directive 2009/54/EC on the exploitation and marketing of natural mineral waters
Parasites		0 (absence)		Directive 2009/54/EC on the exploitation and marketing of natural mineral waters
Pathogens		0 (absence)		Directive 2009/54/EC on the exploitation and marketing of natural mineral waters

1. MICROBIOLOGICAL CRITERIA FOR DRINKING WATER IN EU LEGISLATION

The Directive (Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption[22]) is intended to protect human health by laying down healthiness and purity requirements which must be met by drinking water within the European Union. It applies to all water intended for human consumption apart from natural mineral waters and waters which are medicinal products and establishes strict quality standards for water used for human consumption. Maximum and guideline values for various physical, bacteriological and chemical contaminants are set out.

Table 1: Microbiological criteria for drinking water (Council Directive 98/83/EC)

Parameter	Parametric value (number per 100 ml)
E. coli	0
Enterococci	0
The following applies to water offered for sale in bottles or containers	
E. coli	0/250 ml
Enterococci	0/250 ml
Pseudomonas aeruginosa	0/250 ml
Colony count 22˚C	100/ ml
Colony count 37˚C	20/ ml

2. MICROBIOLOGICAL CRITERIA FOR DRINKING WATER AS SUGGESTED BY WHO

Table 2: Microbiological criteria for drinking water (WHO[23])

Parameter	Parametric value (number per 100 ml)
E. coli or thermotolerant coliform bacteria[23]	0
Total coliform bacteria	0

1. Immediate investigative action must be taken if either *E. coli* or total coliform bacteria are detected. The minimum action in the case of total coliform bacteria is repeat sampling; if these bacteria are detected in the repeat sample, the cause must be determined by immediate further investigation.
2. Although *E. coli* is the more precise indicator of faecal pollution, the count of thermotolerant coliform bacteria is an acceptable alternative. If necessary, proper confirmatory tests must be carried out. Total coliform bacteria are not acceptable indicators of the sanitary quality of rural water supplies, particularly in tropical areas where many bacteria of no sanitary significance occur in almost all untreated supplies.
3. It is recognized that, in the great majority of rural water supplies in developing countries, faecal contamination is widespread. Under these conditions, the national surveillance agency should set medium-term targets for the progressive improvement of water supplies, as recommended in Volume 3 of Guidelines for drinking-water quality.

3. MICROBIOLOGICAL CRITERIA FOR BATHING WATERS

Directive 76/160/EEC of 8 December 1975 concerning the quality of bathing water covers the quality of bathing waters for protecting human health and for reasons of amenity and seeks to ensure that quality is raised over time largely by ensuring sewage is not present or has been adequately diluted or destroyed. Bathing waters are defined as "fresh or sea water in which bathing is explicitly authorised or is not prohibited and is traditionally practised by a large number of bathers".

A new Directive on bathing water (Directive 2006/7/EC) came into force on 24 March 2006 and will repeal the existing 1976 Directive with effect from 31 December 2014. The new Directive establishes

[22] OJ L 330, 5.12.1998, p. 32–54

[23] Assessment and Management of Seafood Safety and Quality, By H.H. Huss, L. Ababouch, L. Gram, Food and Agriculture Organization (FAO) of the United Nations, Rome, 2003 ftp://ftp.fao.org/docrep/fao/006/y4743e/y4743e00.pdf

stricter microbiological standards for two new parameters, Intestinal enterococci and *Escherichia coli*, which will be used to classify bathing waters as 'poor', 'sufficient', 'good' and 'excellent'.

Table 3: Microbiological parameters for coastal and transitional waters (Directive 2006/7/EC)

Parameter	Excellent quality	Good quality	Sufficient	Reference method of analysis
Intestinal enterococci (cfu/100ml)	100(*)	200(*)	185(*)	ISO 7899-1 or ISO 7899-2
E. coli (cfu/100 ml)	250(*)	500(*)	500(*)	ISO 9308-3 or ISO 9308-1

(*) Based upon a 95‑ percentile evaluation. See Annex II of Directive.

4. MICROBIOLOGICAL CRITERIA FOR SHELLFISH WATERS IN EUROPE

Harmonised criteria on the quality required for shellfish waters are established in Directive 2006/113/EC of the European Parliament and of the Council of 12 December 2006[24]. Shellfish waters are those coastal and brackish waters designated by the Member States as needing protection or improvement in order to support (bivalve and gastropod) molluscs life and growth and thus to contribute to the high quality of edible shellfish products. The Directive establishes physical, chemical and microbiological requirements that designated shellfish waters must either fulfil or attempt to improve, as some of which are mandatory while others are guidelines.

Table 4: Microbiological parameters for shellfish waters (Directive 2006/113/EC)

Parameter	Guide	Mandatory	Reference method of analysis	Minimum sampling and measuring frequency
Faecal coliforms/ 100 ml	≤ 300 in the shellfish flesh and intervalvular liquid		Method of dilution with fermentation in 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 ˚C ± 0.5 ˚C	Quarterly

5. MICROBIOLOGICAL CRITERIA FOR SHELLFISH GROWING WATERS IN USA AND CANADA

The National Shellfish Sanitation Program (NSSP) has issued guidelines to identify survey and classify shellfish growing waters[25]. Classification status is based on sanitary surveys of water quality and shoreline surveys of pollution sources. Individual growing areas are classified either as approved for harvest or as one of four harvest-limited categories: (1) conditionally approved, (2) restricted, (3) conditionally restricted, or (4) prohibited. All identified growing waters must be classified as prohibited unless sanitary surveys indicate that water quality meets specific NSSP standards for the other categories.

- Approved: waters from which shellfish may be harvested for direct marketing. Fecal coliform median or geometric mean MPN does not exceed 14 per 100 ml, and not more than 10 percent of the samples exceed an MPN of 43 per 100 ml for a 5-tube decimal dilution test.

[24] OJ L 376, 27.12.2006, p. 14–20

[25] www.fda.gov/Food/FoodSafety/Product-SpecificInformation/Seafood/FederalStatePrograms/NationalShellfishSanitationProgram/ucm046353.htm

- Conditionally approved: waters meeting approved classification standards under predictable conditions. These waters are open to harvest when water quality standards are met, and are closed at other times. Fecal coliform standards are the same as for approved.
- Conditionally restricted: growing waters that sometimes meet the criteria to be restricted; may be harvested if shellfish are subjected to a suitable purification process.
- Restricted: waters from which shellfish may be harvested only if they are relayed or depurated before direct marketing. Fecal coliform median or geometric mean MPN does not exceed 88 per 100 ml, and not more than 10 percent of the samples exceed an MPN of 260 per 100 ml for a 5-tube decimal dilution test.
- Prohibited: waters from which shellfish may not be harvested for marketing under any conditions.

The water quality criteria for shellfish growing areas in Canada (British Columbia)[26] are similar to the US. Faecal coliforms in fresh and marine waters used for the growing and harvesting of shellfish for human consumption should not exceed a median MPN of 14/100 ml over 30 days, and at least 90 % of the samples in a 30-day period should not exceed 43/100 ml. Enterococci in fresh and marine waters used for the growing and harvesting of shellfish for human consumption should not exceed a median MPN of 4/1 00 ml, and at least 90 % of the samples in a 30-day period should not exceed 11/100 ml. In addition, the meat of shellfish must meet a maximum criterion of 230 faecal coliforms/100 g wet weight. The criteria for faecal coliforms are the only ones that apply now.

6. CHEMICAL CRITERIA FOR DRINKING WATER IN EU LEGISLATION

To protect the health of the consumer chemical criteria for the quality of potable water have been laid down in Council Directive 98/83/EC[12] on the quality of water intended for human consumption. The different chemicals and their parametric value are presented in Table 5.

[26] Canadian Council of Resource and Environment Ministers, CCREM. Canadian water quality guidelines. Task Force on Water Quality Guidelines, 1987

Table 5: Chemical parameters laid down in Annex 1, part B, of Council Directive 98/83/EC[12] on the quality of water intended for human consumption.

Parameter	Parametric value	Unit
Acrylamide	0.10	µg/l
Antimony	5.0	µg/l
Arsenic	10	µg/l
Benzene	1.0	µg/l
Benzo(a)pyrene	0.010	µg/l
Boron	1.0	mg/l
Bromate	10[a]	µg/l
Cadmium	5.0	µg/l
Chromium	50	µg/l
Copper	2.0	mg/l
Cyanide	50	µg/l
1,2-Dichloroethane	3.0	µg/l
Epichlorohydrin	0.10	µg/l
Fluoride	1.5	mg/l
Lead	10	µg/l
Mercury	1.0	µg/l
Nickel	20	µg/l
Nitrate	50[b]	mg/l
Nitrite	0.50[b]	mg/l
Pesticides	0.10[c,d]	µg/l
Pesticides - Total	0.50[c,e]	µg/l
Polycyclic aromatic hydrocarbons	0.10[f]	µg/l
Selenium	10	µg/l
Tetrachloethene and Trichloroethene (sum)	10	µg/l
Trihalomethanes - Total	100[g]	µg/l
Vinyl chloride	0.50	µg/l

a) Where possible, without compromising disinfection, Member States should strive for a lower value.
b) Member States must ensure that the condition that [nitrate]/50 + [nitrite]/3 $\leq$ 1, the square brackets signifying the concentrations in mg/l for nitrate (NO_3) and nitrite (NO_2), is complied with and that the value of 0.10 mg/l for nitrites is complied with ex water treatment works.
c) 'Pesticides' means: organic insecticides, organic herbicides, organic fungicides, organic nematocides, organic acaricides, organic algicides, organic rodenticides, organic slimicides, related products (*inter alia*, growth regulators) and their relevant metabolites, degradation and reaction products. Only those pesticides which are likely to be present in a given supply need be monitored.
d) The parametric value applies to each individual pesticide. In the case of aldrin, dieldrin, heptachlor and heptachlor epoxide the parametric value is 0.030 µg/l.
e) 'Pesticides — Total' means the sum of all individual pesticides detected and quantified in the monitoring procedure.
f) The specified compounds are benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, and indeno(1,2,3-cd) pyrene.
g) Where possible, without compromising disinfection, Member States should strive for a lower value. The specified compounds are: chloroform, bromoform, dibromochloromethane, bromodichloromethane

7. CHEMICAL CRITERIA FOR NATURAL MINERAL WATER IN EU LEGISLATION

The protect the health of the consumer quality criteria have been laid down in Commission Directive 2003/04/EC,[13] establishing the list, concentration limits and labelling requirements for the constituents of natural mineral waters. The maximum limits, which, if exceeded, may pose a risk to public health as laid down in Annex 1 of this Directive are presented in Table 6.

Table 6: Maximium limits for constituents naturally present in natural mineral waters as laid down in Annex 1 of Commission Directive 2003/04/EC. [13]

Constituents	Maximum limits (mg/l)
Antimony	0.0050
Arsenic	0.010 (as total)
Barium	1.0
Boron	For the record (*)
Cadmium	0.003
Chromium	0.050
Copper	1.0
Cyanide	0.070
Fluoride	5.0
Lead	0.010
Manganese	0.50
Mercury	0.0010
Nickel	0.020
Nitrates	50
Nitrites	0.1
Selenium	0.010

(*) The maximum limit for boron will be fixed, where necessary, following an opinion of the European Food Safety Authority and on a proposal from the Commission by 1 January 2006.

B. TREATMENTS OF SEAWATER

1. WATER TREATMENT METHODS

There are several treatment methods used to produce safe and clean water, and to remove/prevent unpleasant taste and odour. The main treatment methods will be described briefly below. Preliminary treatment of water by coagulation settlement or sand filtration will remove turbidity that may interfere with many of the disinfection processes. A summary of water treatment methods is given in a technical manual published by the Scottish Executive (2006[27]). Some additional considerations with regard to seawater are given in a manual on bivalve depuration published by FAO (2008).

1.1 Chemical coagulation

Chemical coagulation is the most common approach for treatment of surface waters. Salts of aluminium or iron, are dosed to the source water to remove suspended and dissolved contaminants. The efficiency of the coagulation process depends on the raw water quality, the coagulant used and operational factors, including mixing conditions, coagulation dose and pH. The floc which is formed is removed from the treated water by subsequent processes such as sedimentation or filtration. Coagulation is suitable for removal of particulates and bound microorganisms, certain heavy metals and low-solubility organic chemicals, such as certain organochlorine pesticides. For other organic chemicals, coagulation is generally ineffective, except where the chemical is adsorbed to humid material or particulates.

1.2 Sand filtration

Slow sand filters are used to remove algae and microorganisms, including protozoa, and, if preceded by microstraining or coarse filtration, to reduce turbidity, including adsorbed chemicals. Slow sand filtration is also effective for the removal of some organic compounds, including certain pesticides.

1.3 Microfiltration

Microfiltration uses membranes that can exclude particles that are larger than 0.05µm. This means that bacteria, protozoal cysts and algae will be removed from the water although viruses may pass through. Salt molecules will cross the membrane and so the process is applicable to seawater. Membranes with smaller pore sizes, e.g. those used for nanofiltration, will also exclude viruses.

1.4 Ultraviolet irradiation

Ultraviolet (UV) disinfection is achieved by passing water through units containing lamps that have their main output in the UVc region of the spectrum (200 to 280nm; peak microbiocidal wavelength 254nm). There are two main types of lamp: low pressure and medium pressure. The latter are used for high-throughput systems. The design of unit may vary but smaller units will use a UV-producing tube within a quartz sleeve with the water passing down the space between the tube and the sleeve. The UV dose that is received by the target microbes will depend on the output of the unit, the flow rate of the water through the unit and the transmissivity of the water (ability for UV to pass through). Much higher doses of UV are required to inactivate viruses than bacteria.

The efficiency of UV output in the target range decreases with use. Manufacturers generally specify lifetimes that equate to a remaining efficiency of 80 percent of the original. It is the output at the end of the rated life-time that should be used in determining the size of a UV unit needed for a specific system. The transmissivity depends on several factors, including the turbidity of the seawater and the presence of dissolved inorganic salts or organic material. If a quartz-sleeve system is used, the amount of UV light reaching the water will also depend on the state of cleanliness of that sleeve. UV dosage can be quoted as either the applied dose (usually calculated from the output of the lamp - either theoretical or measured) and the transmissivity of the water, or as the received dose (actually measured

[27] Scottish Executive, 2006. Private Water Supplies:Technical Manual. Section 6. Water Treatment Processes. Edinburgh, Scottish Executive.

at the wall of the tube containing the lamp). In practice, accurate measurement of received UV dose has proved to be difficult to achieve.

1.5 Chlorination

Chlorination is achieved by adding chlorine gas, chlorine dioxide, sodium hypochlorite, calcium hypochlorite or monochloramine. With seawater, it may also be generated in situ using electrolysis. The disinfection efficiency is affected by the following factors: concentration of available chlorine, degree of mixing, contact time, pH, water temperature, turbidity and interfering substances. Chlorine combines with any ammonia in the water to produce chloramines. It is mainly the free chlorine, and to a lesser extent the monochloramine, which cause the disinfection.

Chlorine is effective against bacteria but less so against some viruses. The infective stage of some parasites, such as the cysts of *Giardia duodenalis* and, to a greater extent, the oocysts of *Cryptosporidium* spp., are resistant to chlorine at the normal concentrations used for the disinfection of drinking water.

The effectiveness of chlorination is determined using CT values. The CT value is calculated by multiplying the concentration of free chlorine in mg/l by the contact time in minutes. Usually, a minimum CT value of 6 is targeted (e.g. at least 0.2 mg/l free chlorine for 30 minutes) in order to achieve disinfection of bacteria and viruses. This assumes that the other factors such as pH and temperature are optimal. Maintenance of a concentration of residual free chlorine after treatment ensures that bacterial regrowth does not occur.

The possible formation of potentially toxic compounds such as chloramines or other by-products when adding chlorine to seawater should be considered. Chlorination of water containing organic material will result in the production of by-products such as trihalomethanes (THM) and halogenated acetic acids (HAA). Treatment of water prior to chlorination can minimize toxic by-product formation.

Free chlorine in water is usually measured using a method based on diethyl paraphenylene diamine (DPD). This chemical is oxidised by chlorine to produce a coloured compound. Total chlorine can be measured by adding potassium iodide to the reaction after the free chlorine result has been recorded. Continuous chlorine monitors are available for process control.

1.6 Ozonation

Ozone is very effective at inactivating both bacteria and viruses. It is also fairly effective against the oocysts of *Cryptosporidium*. It may be purchased as the gas form in cylinders or produced on-site by means of high energy electrical discharge or UV light (peak wavelength at 185 nm rather than the 254 nm used for UV disinfection). The ozone is then introduced into the water via a diffuser in order to get good mixing. Ozone is a relatively expensive form of disinfection and the gas is very toxic.

In seawater, ozone may oxidise any bromide to bromate, which is of concern as a carcinogen. To minimize this effect, ozone should not be used with a concentration exceeding 0.5 mg/l (the usual concentration is approximately 0.4 mg/l).

Although ozone breaks down rapidly, ozonation is applied, e.g. in bivalve mollusc purification systems. For this application, ozonization is undertaken in a separate tank to that used for depuration and then the residual ozone has to be discharged from the seawater before use so that it does not adversely affect the animals – this is achieved by forced aeration or activated carbon filtration. Where a continued bactericidal effect is required, supplementary low-level chlorination may be applied.

1.7 Distillation

Distillation is used to produce some bottled drinking waters. The resulting water is free from micro-organisms and other impurities. Ice for use in drinks may also be made from such water. The process

is obviously not applicable to the production of disinfected seawater as the product will not contain any sea salt.

1.8 Reverse osmosis

Reverse osmosis is the flow of solute through a semi-permeable membrane from a higher concentration to a lower concentration by applying pressure to the high concentration side. Membrane pore sizes are less than 0.002 μm and so micro-organisms will be excluded. However, salt molecules are also retained by the membrane and so the process is not applicable to seawater.

1.9 Activated carbon

Activated carbon is normally used as powdered activated carbon (PAC) or in granular form (GAC). GAC has a high affinity for organic compounds. It is normally used in fixed beds, either in absorbers for specific chemicals or in sand filters to replace sand with GAC of a similar particle size. It is common practice to install GAC absorbers between the filtration and the disinfection steps. Different types of GAC vary considerably in their capacity for specific organic compounds, which can have a significant effect upon their service life. GAC is used for the removal of pesticides and other organic chemicals, taste and odour compounds, cyanobacterial toxins and total organic carbon.

1.10 Ion exchange

Ion exchange is a process in which ions are exchanged between the water and the solid resin phase. Ion exchange can be used to remove contaminants such as nitrate, boron, fluoride, or uranium. Several resins are available for this purpose

2. Application of disinfection methods to the disinfection of seawater

Most bottled waters are taken from springs or groundwater sources and, in general, the source water tends to have low levels of particulate and organic matter. Such sources may be sufficiently protected to be consumed without any treatment. Seawater however may contain significant amounts of particulate and organic matter. The levels of these may vary with location, tidal state and season. Depending on the geographical area, seawater may also contain significant levels of pathogens, either naturally occurring (such as some *Vibrio* spp.) or derived from faecal contamination (e.g. epidemic *V. cholerae*, *Salmonella*, norovirus). There is therefore the need to consider the location and timing of extraction of seawater for treatment so that the subsequent processes are able to produce a good quality safe product. The application of methods such as settlement or sand filtration will remove material that will interfere with the disinfection processes. Lastly, there may be the need to use additional steps such as activated carbon filtration that will remove other contaminants or disinfection by-products. Some water treatment systems include a series of treatment steps: sand filtration, ozonation, activated carbon filtration and then UV.

3. Monitoring of disinfection processes

Turbidity can be monitored continuously to ensure that it is less than the critical limit defined for the process. With UV systems, there is a need to ensure that the lamps are functioning and within their specified lifetime. Chlorination and ozonation processes can be monitored continuously online and samples can also be taken for chemical confirmation of the disinfectant concentration. Membrane processes such as reverse osmosis and microfiltration can be monitored by pressure differential across the membrane or turbidity measurement or particle counting of the filtrate: satisfactory results in these tests may not mean that pathogens have not passed through the membrane due to imperfections.

Microbiological monitoring is usually based on a combination of bacterial viable counts and faecal indicator testing. These may be supplemented with tests for specific pathogens.

Table 7: Monitoring criteria for water treatments

	Monitoring criteria		
Disinfection method	**Pre-disinfection (post-settlement or filtration)**	**Disinfection**	**Post-disinfection**
Microfiltration	Turbidity	Pressure across membrane (usually 1-2 bar)	Turbidity or particle analysis
UV irradiation		Flow, lamps working, applied dose Lamp-life log	
Chlorination		pH Free chlorine and/or monochloramine concentration Contact time	Residual chlorine concentration
Ozonation		Redox potential Ozone concentration	Ozone concentration

C. OUTLINE DESCRIPTIONS OF STANDARD METHODS

Parameter	Method	Outline descriptions
Escherichia coli	EN ISO 9308-3	**Water Quality – Detection and enumeration of *Escherichia coli* and coliform bacteria in surface and waste water – Part 3**. This method provides a most probable number estimation of the numbers of *E. coli* by inoculation of water samples into a liquid medium. The method is applicable to all types of surface and waste waters (including seawater), particularly those rich in suspended matter. Diluted samples and inoculated into microtitre plate wells containing dehydrated MUG/EC medium (methylumberlliferyl-β-D-glucuronide). Following incubation at 44 °C for 36-72 hrs, the presence of *E. coli* is indicated by blue fluorescence resulting from hydrolysis of the MUG detected in a UV observation chamber.
Escherichia coli	EN ISO 9308-1	**Water Quality – Detection and enumeration of Escherichia coli and coliform bacteria – Part 1**. This method is based on membrane filtration of water samples, subsequent culture on a differential agar medium and calculation of the number of target organisms in the sample. This method provides a rapid method for the detection of *E. coli* within 24 hrs which is applicable to waters with low bacterial numbers or a standard method which takes 2-3 days. The standard and rapid methods can be applied to waters provided suspended matter or background flora does not interfere with filtration, culture and counting. The standard method involves incubation of the membrane on selective lactose agar, incubated at 36 °C for 21 hrs. For the rapid test involves incubation of the membrane on casein (tryptic digest) agar, incubated at 36 °C for 4-5 hrs followed by incubation 44 °C for 20 hrs on a medium containing casein (tryptic digest) agar containing bile salts. Characteristics lactose fermenting colonies are counted and randomly selected for subculture and confirmation using oxidase and indole production.
Enterococci	EN ISO 7899-1	**Water quality – Detection and enumeration of intestinal enterococci in surface and waste water – Part 1.** This method provides a most probable number estimation of the numbers of enterococci by inoculation of water samples into a liquid medium. The method is applicable to all types of surface and waste waters (including seawater), particularly those rich in suspended matter. Diluted samples and inoculated into microtitre plate wells containing dehydrated MUD medium (4-methylumberlliferyl-β-D-glucoside) containing tallium acetate, nalidixic acid and triphenyltetrazolium chloride. Following incubation at 44 °C for 36-72 hrs, the presence of enterococci is indicated by fluorescence resulting from hydrolysis of the MUD detected in a UV at 366 nm.
Enterococci	EN ISO 7899-2	**Water quality – Detection and enumeration of intestinal enterococci – Part 2.** This method is based on membrane filtration of water samples, subsequent culture on a differential agar medium and calculation of the number of target organisms in the sample. The method can be applied to all types of waters (including seawater) except when a large amount of suspended matter or interfering micro-organisms are present. The method involves incubation of the membrane on to selective agar containing sodium azide and, triphenyltetrazolium chloride incubated at 36 °C for 44 hrs. Characteristics colonies are counted and the membrane is transferred to bile-aesculin agar preheated to 44 °C. Enterococci hydrolyse the aesculin within 2 hrs.

Parameter	Method	Outline descriptions
Vibrio	ISO/TS 21872-1:2007	**Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. -- Part 1: Detection of *Vibrio parahaemolyticus* and *Vibrio cholera*** This part of ISO/TS 21872 specifies a horizontal method for the detection of the two main pathogenic *Vibrio* species causing intestinal illness in humans: *V. parahaemolyticus* and *V. cholerae*. It is applicable to products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling. The detection of *Vibrio parahaemolyticus* and *Vibrio cholerae* requires four successive phases including two enrichments in a liquid selective broths, isolation and identification of presumptive colonies using two solid selective media (thiosulfate citrate bile and sucrose agar, TCBS, and another appropriate solid selective medium) and a final confirmation by means of appropriate biochemical tests.
Vibrio	ISO/TS 21872-2:2007	**Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. -- Part 2: Detection of species other than *Vibrio parahaemolyticus* and *Vibrio cholerae*** This method specifies a horizontal method for detection of the enteropathogenic *Vibrio* species, causing illness in or via the intestinal tract, other than *Vibrio parahaemolyticus* and *Vibrio cholerae*. The species detectable by the methods specified include *Vibrio fluvialis*, *Vibrio mimicus* and *Vibrio vulnificus*. It is not suitable for the isolation of *Vibrio hollisae*. Strains of *V. parahaemolyticus* and *V. cholerae* may also be detected during the application of this method. ISO/TS 21872-2:2007 is applicable to products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling. This method is not appropriate for the detection of *Vibrio metschnikovii* as this is oxidase negative

GLOSSARY

Regulation (EC) No 852/2004 defines:

'Clean seawater' as "natural, artificial or purified seawater or brackish water that does not contain micro-organisms, harmful substances or toxic marine plankton in quantities capable of directly or indirectly affecting the health quality of food"

'Clean water' means clean seawater and fresh water of a similar quality;

'potable water' means water meeting the minimum requirements laid down in Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption (1);

COUNCIL DIRECTIVE 98/83/EC defines:

'water intended for human consumption' shall mean:

a) all water either in its original state or after treatment, intended for drinking, cooking, food preparation or other domestic purposes, regardless of its origin and whether it is supplied from a distribution network, from a tanker, or in bottles or containers;

b) all water used in any food-production undertaking for the manufacture, processing, preservation or marketing of products or substances intended for human consumption unless the competent national authorities are satisfied that the quality of the water cannot affect the wholesomeness of the foodstuff in its finished form.

Regulation (EC) No 853/2004 defines:

'Fishery products' means all seawater or freshwater animals (except for live bivalve molluscs, live echinoderms, live tunicates and live marine gastropods, and all mammals, reptiles and frogs) whether wild or farmed and including all edible forms, parts and products of such animals.

'Fresh fishery products' means unprocessed fishery products, whether whole or prepared, including products packaged under vacuum or in a modified atmosphere, that have not undergone any treatment to ensure preservation other than chilling.

'Prepared fishery products' means unprocessed fishery products that have undergone an operation affecting their anatomical wholeness, such as gutting, heading, slicing, filleting, and chopping.

Code of practice for fish and fishery products (CAC/RCP 52-2003) defines:

Clean water means water from any source where harmful microbiological contamination, substances and/or toxic plankton are not present in such quantities that may affect the safety of fish, shellfish and their products intended for human consumption.

Codex General Standard for quick frozen fish fillets (CODEX STAN 190 – 1995) defines:

Clean seawater is seawater which meets the same microbiological standards as potable water and is free from objectionable substances.

Monitoring: The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control.

Sanitary survey: survey of the faecal pollution inputs, and their potential circulation within a given marine environment

Validation: Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.

Verification: The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended.

ABBREVIATIONS

ADI	Acceptable daily intake
ASP	Amnesic shellfish poisoning
B2M	Beta-2-microglobulin
BIOHAZ Panel	EFSA Panel on Biological Hazards
BMD	Benchmark dose
$BMDL_{01}$	Benchmark dose (BMD) of 1 % extra risk
$BMDL_5$	Benchmark dose lower confidence limit
B-Pb	Blood lead
BTX	Brevetoxin
b.w.	Body weight
Cd	Cadmium
CKD	Chronic kidney disease
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
Cu	Copper
DA	Domoic acid
DPD	Diethyl paraphenylene diamine
DSP	Diarrhoeic shellfish poisoning
GAC	Granular activated carbon
HAA	Halogenated acetic acids
IARC	International Agency for Research on Cancer
ICES	International Council for the Exploration of the Sea
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO meeting on Pesticides Residues
MOE	Margin of exposure
NDA	EFSA Panel on Dietetic Products, Nutrition, and Allergies
NOAEL	No-observed-adverse-effect level
NSP	Neurotoxic shellfish poisoning
OA	Okadaic acid
PAC	Powdered activated carbon
PAHs	Polynuclear aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
PSP	Paralytic shellfish poisoning
PTWI	Provisional tolerable weekly intake
PlTX	Palytoxin
ROS	Reactive oxygen species
SBP	Systolic blood pressure

SCF	Scientific Committee on Food
STX	Saxitoxin
TDI	Tolerable Daily Intake
THM	Trihalomethanes
UL	Upper intake level
U.S.A.	United States of America
UV	Ultraviolet
V	Antimony
WHO	World Health Organization