

Maisons-Alfort, 26 July 2007

OPINION

of the French Food Safety Agency concerning the establishment of hygiene rules for using clean seawater in the handling of fishery products

1. REVIEW OF THE REQUEST

The French Food Safety Agency (AFSSA) received a request on 7 November 2006 from the Directorate General for Food (DGAL) for an opinion concerning the establishment of hygiene rules for using clean seawater in the handling of fishery products.

2. QUESTIONS POSED

The Agency was requested to respond to the following questions:

Question 1: Based on the parameters applicable to water intended for human consumption, which ones would be appropriate to retain for clean seawater used in the handling of fishery products? Can other criteria be proposed? What values can be proposed?

Question 2: Concerning toxic marine plankton, can indicative threshold values be proposed?

Question 3: What are the definitions of pumping areas for seawater "exposed" and "unexposed" to microbiological and/or physico-chemical contamination? List the characteristics for each of these designations in relation to the parameters defined in Question 1, as well as the criteria relating to geography (distance to coast, depth of pumping), climate and tide.

Question 4: Is it appropriate to define several quality levels of clean seawater according to its uses in different types of fishery products?

3. BACKGROUND

The use of clean seawater in the handling of fishery products has been regulated since 1 January 2006 by Regulations (EC) No. 852/2004 and 853/2004¹.

According to Regulation (EC) No. 853/2004, live bivalve molluscs, live echinoderms, live tunicates and live marine gastropods are excluded from the definition of fishery products.

According to Regulation (EC) No. 852/2004, clean seawater is *"natural, artificial, or purified seawater or brackish water that does not contain microorganisms, harmful substances, or toxic marine plankton in quantities capable of directly or indirectly affecting the health quality of food."*

¹ Regulation (EC) No. 852/2004 of 29 April 2004 on the hygiene of foodstuffs.
Regulation (EC) No. 853/2004 of 29 April 2004 laying down specific hygiene rules for food of animal origin.

Clean seawater is used for the following purposes:

- 1) Water supply for fish and crustacean tanks
- 2) Washing and cooling of crustaceans and molluscs after cooking
- 3) Handling and washing of unprocessed products such as fish fillets and slices
- 4) Washing of whole, gutted, and beheaded fishery products
- 5) Manufacture of ice for cooling and storage of fishery products, fresh or processed
- 6) Cleaning of facilities and equipment.

Depending on the nature of the water used at fish auctions, and to the extent of information provided to the Agency, three situations can be distinguished:

- 1) Fish auctions that no longer use seawater
- 2) Those using saline groundwater
- 3) Those using seawater, of which only a small number would cook crustaceans in seawater.

It should be noted that an inventory of uses of seawater by fish auctions and shore establishments should be undertaken by the competent authorities.

Regulation (EC) No. 853/2004 restricts the use of clean seawater to whole products, and, on board ships, to gutted and beheaded fishery products. However, transitional provisions allow it to be used, until 31 December 2009, for all other purposes (manufacture of ice and handling of fishery products in the shore establishments and fish auctions, and cooling of cooked crustaceans and molluscs).

However, the French authorities wish to perpetuate the use of clean seawater for the above-mentioned purposes, subject to strict compliance by the involved fishery workers with health requirements, through appropriate modification of EU legislation.

Consequently, the French authorities must develop technical and scientific arguments during this transitional period, for the attention of the European Commission. In this context, the French Food Safety Agency has been asked to propose quality criteria for clean seawater, which are currently nonexistent. It should be noted that, in addition to the definition of these criteria, this request will provide the competent authorities with ways of generating data by 2009.

The fishery workers themselves have to propose and implement the means to ensure quality control of the seawater they use in their institutions (through good practice guides, for example).

4. EXPERT APPRAISAL METHOD

Two Expert Committees (CES) from AFSSA were involved in this expert appraisal: the CES on Microbiology (MIC) and the CES on Physical and Chemical Contaminants and Residues (RCCP), as well as experts from the CES on Water, and external experts.

Various documents (regulatory texts, documents relating to New Zealand legislation, to the composition of seawater, to the various quality initiatives undertaken by certain countries, documents from the *Codex Alimentarius*, WHO, FAO, results from analysis and own checks provided by the DGAL), were made available to the experts.

All documents used as part of this expert appraisal are referenced in Section 9, 'Main Bibliographic References,' below.

Following the consultations with the CES on Microbiology, held on 8 February 2007, 20 March 2007 and 22 May 2007, and the CES on Physical and Chemical Contaminants and Residues on 21 March 2007, AFSSA issued the following opinion:

5. FIELD OF EXPERTISE

The risk assessment undertaken for this Opinion is strictly limited to the use of clean seawater in the handling of fishery products in shore establishments and fish auctions; therefore, the uses of seawater excluded from this request are:

- in shellfish farms
- in fish farms
- on ships.

6. ADVANTAGES AND DISADVANTAGES OF SEAWATER USE

The use of seawater in fish auctions and shore establishments in the handling of fishery products has the following advantages:

- seawater is an advantageous resource because it is found close to the facilities, it is inexpensive, and its supply is unlimited, in contrast to drinking water;
- seawater does not cause osmotic shock in seafood or coagulation of mucus on fish skin; marine organisms should also be cooked in salt water.

Among the principal disadvantages, it should be noted that salt water heavily corrodes facilities (walls, floors, etc.) and metal equipment. In addition, salt wastewater can damage treatment systems for domestic wastewater due to its salinity, which requires specific purification systems to be set up in establishments.

7. ELEMENTS FOR THE CHARACTERISATION OF CLEAN SEAWATER

Three main types of hazards are to be considered when characterising clean seawater:

- 1) Microbiological hazards
- 2) Chemical contaminants (inorganic and organic)
- 3) Marine phycotoxins.

The influence of general parameters (such as pH, temperature, salinity, and turbidity) on the behaviour of microbiological and chemical hazards will be examined.

With regard to microbiological hazards

Various types of microbiological hazards may be present in seawater, and contaminate fishery products. Three main types of microbiological hazards are likely to contaminate fishery products:

- bacteria
- viruses
- parasites

Annex 1 of this document presents a detailed description of these potential contaminants, emphasising the following hazards:

- Bacteria:
 - o *Salmonella*
 - o *Listeria monocytogenes*
 - o *Staphylococcus aureus*
 - o *Vibrio*
 - o indicators of contamination: indicators of pollution and treatment efficacy: coliforms, indicators of faecal contamination (*Escherichia coli*, faecal enterococci)
- Viruses:
 - o viral hazard and fishery products
 - o viral contamination of seawater
 - o regulation and monitoring of viral presence/seawater pumping areas
- Parasites:
 - o overview of parasites of interest
 - o elements concerning the evaluation of parasite risk
 - o disinfection procedures and their efficacy on parasites: UV ozone effect on parasites
 - o parasite detection

Considering that:

- within the limits of available information, the uses of seawater have not resulted in any cases being notified indicating that such uses are causing public health problems;
- current European legislation allows the marketing of shellfish grown in seawater for which a tolerance relative to *E. coli* has been established;

Considering in this connection that the European regulation establishing microbiological criteria for fishery products is currently undeveloped, and the CES on Microbiology will issue an opinion soon on microbiological criteria for process hygiene indicators proposed by the concerned industries; that these criteria will have to be listed in Guides to Good Hygiene Practice and the application of Hazard Analysis Critical Control Point (HACCP) principles;

The CES on Microbiology:

- considers that the microbiological requirements for seawater should be similar to those recommended for fishery products themselves, and not those recommended for drinking water;
- recommends that the use of seawater be possible for all the above-mentioned purposes, provided that its use has been subject to an authorisation application which includes the following elements:
 - 1) A preliminary study of the composition of raw seawater at the pumping point and its possible variations, mainly concerning *E. coli* and turbidity, so that under no circumstances will the microbiological quality be lower than that recommended for shellfish farming.²;
 - 2) Risk assessment for the degradation of water quality;
 - 3) A study concerning the vulnerability of the resource³ and the protective measures to be implemented;
 - 4) The rationale for treatment products and processes to be used if necessary (reducing turbidity by retention and lowering the microbial load by disinfection) to be accompanied by a demonstration of the safety and efficacy with regard to the quality of seawater to treat. In cases when exchange resins or activated carbon are used for chemical treatment of water, the usual precautions should be taken to avoid increasing the microbial load of the water;
 - 5) Description of facilities for water production and distribution;
 - 6) Description of the procedures for monitoring water quality (own checks of raw seawater and treated seawater).
- recalls that, in the current state of knowledge and in the absence of public health objectives set by the health authorities, the CES on Microbiology is not able to establish microbiological criteria for seawater used for the above-mentioned purposes,
- recommends that the following additional data concerning the microbiological component be generated (see Annex 1):
 - 7) data on the 'Vibrio' hazard, notably via studies in local fish auctions to better understand seawater contamination levels; similarly, it would also be interesting to carry out local studies on *L. monocytogenes*;
 - 8) environmental studies on seawater and fishery products concerning aspects of virology, parasitology, and bacteriology, in order to determine the presence of these microorganisms in these matrices. It will be appropriate then to determine whether it is pertinent to screen for them once analytical methods are routinely available.

² Directive 2006/113/EC of the European Parliament and the Council of 12 December 2006 on the quality required of shellfish waters (indicating in particular 'faecal coliforms/100 mL: ≤ 300 in shellfish flesh and intervalvular liquid') and Memorandum DGAL/SDSSA/N2003-8058 of 27 March 2003 on conditions for issuing health licenses to shellfish farming centres - supply and use of seawater pumped in area B, indicating in particular "this memorandum introduces indicators of compliance for pumped seawater in order to obtain clean seawater: such water must have:

- a concentration less than 15 *E. coli* in 100 ml according to the NF ISO 9308-3 (NPP) standardised method [or according to another standardised or validated method such as the NF ISO 9308-1 Standard] and
 - absence of salmonella in 5 litres according to ISO method 6340."

³ Such as the influence of river flow in the vicinity of the pumping point.

The conclusions reached by the CES on Microbiology are presented schematically in **Figure 1**.

With regard to chemical contaminants

Chemical contaminants are not directly regulated in seawater. In contrast, fishery products are subject to Regulation (EC) No.1881/2006⁴ which sets maximum levels for lead, cadmium, mercury, dioxins and dioxin-like PCBs, and polycyclic aromatic hydrocarbons (PAHs).

In seawater, chemical contaminants are present either in dissolved form, or in particulate form adsorbed on suspended materials, the latter being the large majority.

Hydrophobic compounds (PCBs, dioxins, PAHs, tributyltin (TBT)), which are the chemical contaminants most likely to contaminate fish during handling with clean seawater, have very low water solubility. Consequently, their levels in seawater are generally very low. For example, PCB levels in filtered seawater are often of the order of pg/L (OSPAR Quality Status Report 2000). In addition, the introduction of adsorption treatment with activated carbon ensures their retention.

Accordingly, the CES on Physical and Chemical Contaminants and Residues does not consider it appropriate to retain all the chemical parameters⁵ applicable to water intended for human consumption in order to define quality criteria for clean seawater, and recommends in the following two situations:

- pumping seawater from the open sea with a turbidity > 1 FNU
- pumping coastal seawater

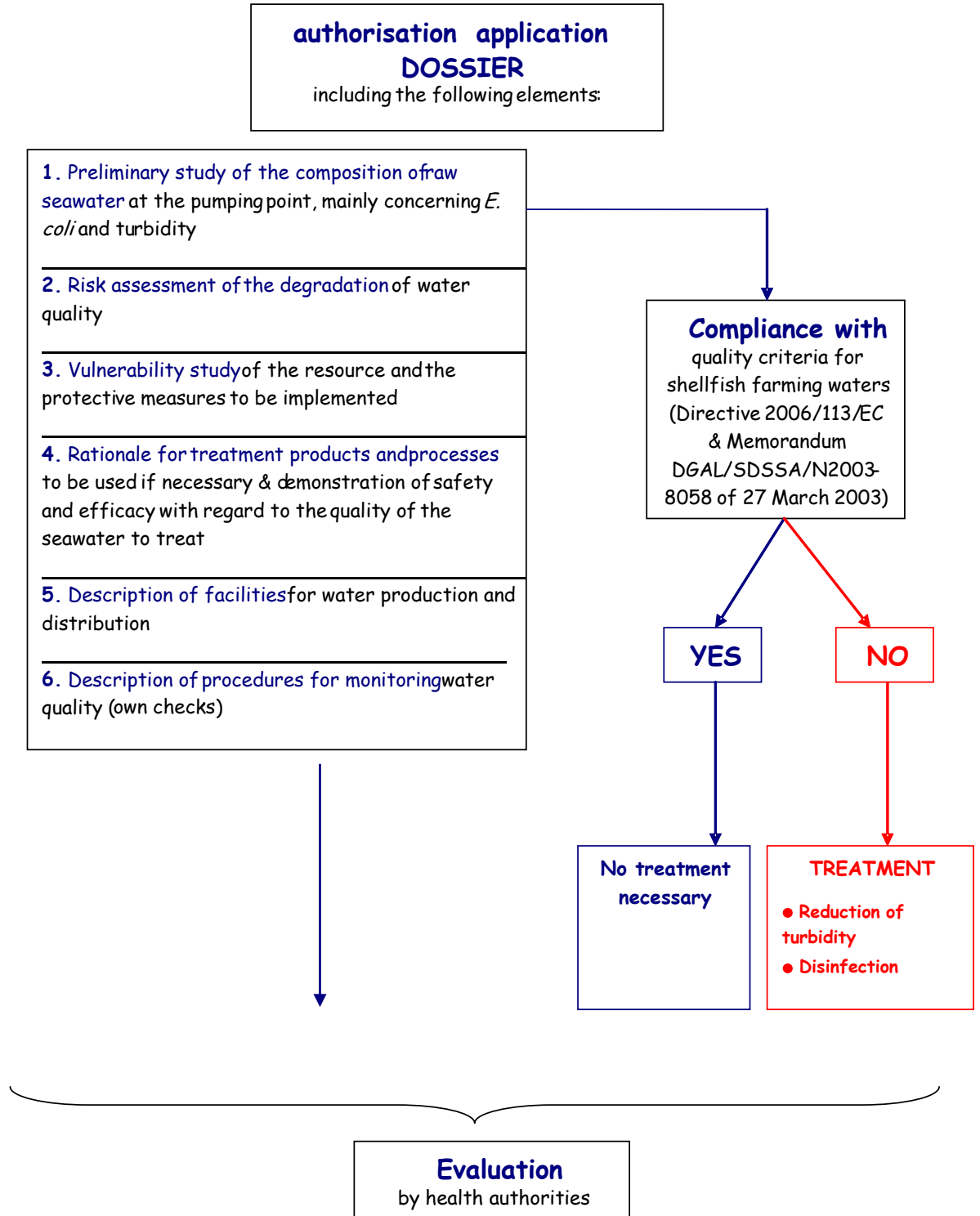
the installation of retention and adsorption treatments, to ensure the health and safety of consumers of fishery products.

⁴ Regulation (EC) No.1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

⁵ Ministerial Order of 11 January 2007 concerning the limits and quality references of raw water and water intended for human consumption, referred to in Articles R. 1321-2. R. 1321-3. R. 1321-7 and R. 1321-38 of the French Public Health Code.

Figure 1:

Summary of the expert appraisal undertaken by the CES on **Microbiology concerning the establishment of hygiene rules for using clean seawater in the handling of fishery products.**



With regard to phycotoxins

The presence of blooms of certain genera or species of phytoplankton in coastal waters can have two distinct types of impact. The most frequent on the French coast is the development of toxic species that can accumulate in filter-feeding shellfish and cause food poisoning in consumers. Additionally, certain potentially harmful species found on the French coast can cause damage and/or massive mortality in fish and other marine organisms by direct contact, without the phenomenon of bioaccumulation, for example by releasing haemolysins.

Toxic phytoplankton

In France, thresholds of phytoplankton cells in seawater have been defined for each of the genera (*Dinophysis*, *Alexandrium*, *Pseudo-Nitzschia*) in order to trigger control of the level of bioaccumulated toxins in shellfish. These thresholds are indicators of a potential risk to shellfish consumers, who may be exposed to a quantity of toxins accumulated by the shellfish over several days. Apart from the case of fish and crustacean tanks, for which no bibliographic data are available, the use of seawater in fish auctions and shore establishments cannot cause the phenomenon of toxin bioaccumulation. However, it is conceivable that a certain number of toxic phytoplankton cells or toxins (if the cells are lysed) may be deposited on products in contact with water, without toxin levels reaching those observed through bioaccumulation.

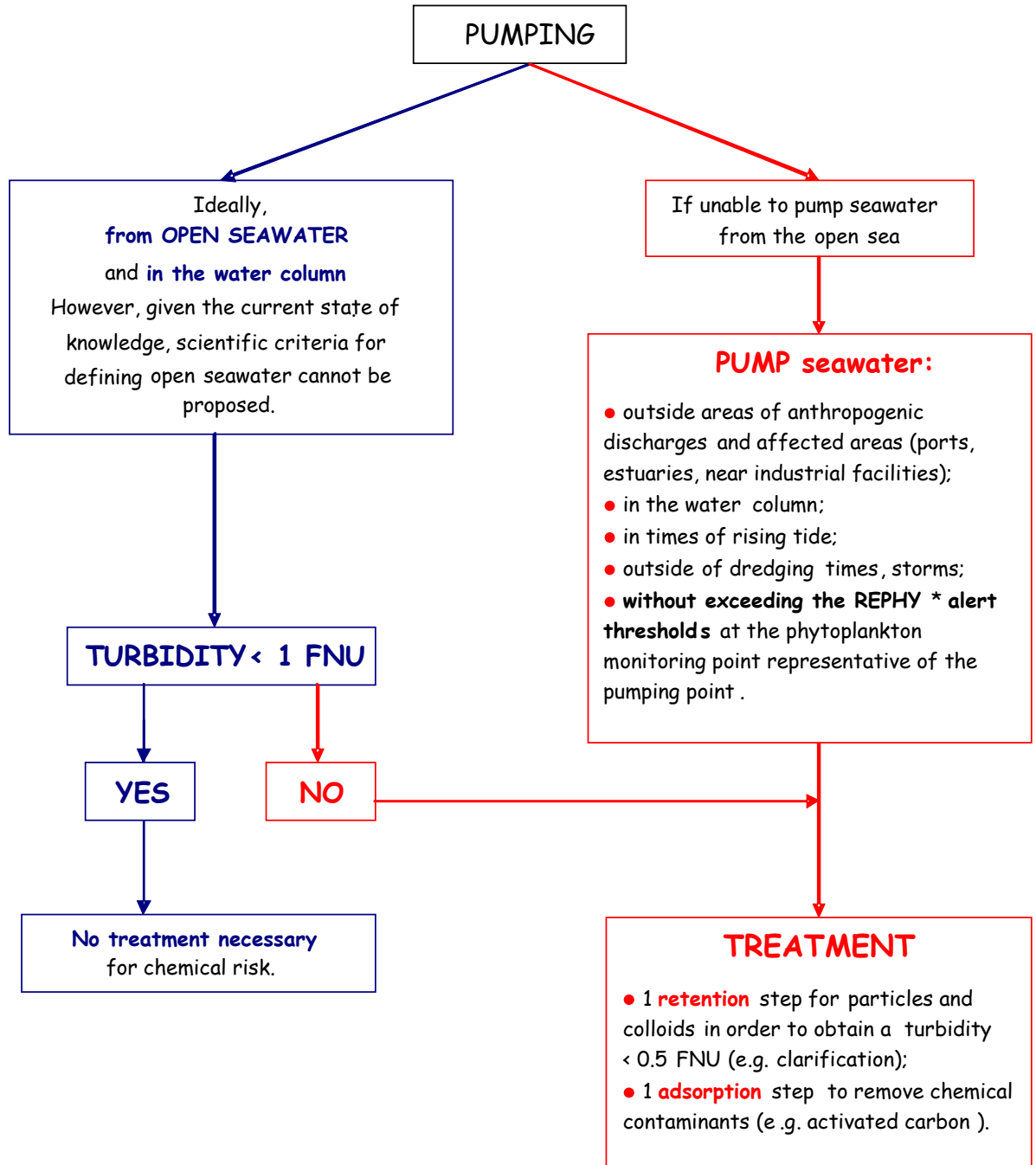
Harmful phytoplankton

Harmful species (e.g., some species of the genera *Gymnodinium* and *Gyrodinium*) cause damage or even death of aquatic organisms by anoxia of the environment, by mechanical effects, or by release of toxins. There are very few data on the risk these toxins may pose to humans when swallowed or by contact during handling. In addition, massive fish damage can significantly affect the overall quality of the surrounding water.

The conclusions reached by the CES on Physical and Chemical Contaminants and Residues are presented schematically in **Figure 2**.

Figure 2:

Summary of the expert appraisal undertaken by the CES on **Physical and Chemical Contaminants and Residues** concerning the establishment of hygiene rules for using clean seawater in the handling of fishery products.



*Unless after further analysis the REPHY alert is proved to correspond to a non-toxic species or genera.

REPHY is the French monitoring network for phytoplankton and phycotoxins

8. AFSSA CONCLUSIONS AND RECOMMENDATIONS

The critical points raised by the Expert Committees consulted in relation to the issue of qualification of clean seawater for the handling of fishery products in shore establishments and at fish auctions are specifically:

- the pumping conditions
- parameters that could justify the introduction of treatment for the seawater used
- phycotoxins
- monitoring of the quality of clean seawater.

1. *Pumping conditions*

Except in the case of scientifically documented local conditions, AFSSA recommends pumping seawater from outside areas of anthropogenic discharge, deep (in the water column), in times of rising tide and not ebb tide, and away from any dredging operation or major storm.

Consequently, AFSSA recommends not pumping seawater in areas frequently polluted by chemical contaminants, such as estuarine waters, port waters⁶ and those located near industrial plants.

The criteria adopted by the OSPAR Convention for periodic assessments of the water status of the convention area could be included in the definition of the clean seawater pumping area, notably the Ecotoxicological Assessment Criteria (EAC), corresponding to concentrations below which adverse biological effects are considered minimal (**Annex 2**).

2. *Parameters that could justify the introduction of treatment for the seawater used*

Considering that it is necessary to take into account the initial quality of the seawater used in the light of potential physico-chemical and microbiological contamination (particularly via turbidity and the *E. coli* parameter);

Considering that the quality criteria for shellfish farming waters, as defined by Directive 2006/113/EC and the Memorandum DGAI/SDSSA/N2003-8058 of 27 March 2003:

- do not take into account the parasitic hazards;
- do not provide quantified parameters for chemical contaminants or suspended particulate materials;

Considering that, given the current state of knowledge, scientific criteria for defining seawater from the open sea cannot be proposed;

Considering that in practice, coastal seawater would most likely be pumped;

Considering that it is unrealistic in practice to develop differential recommendations according to the different proposed uses for seawater, even if the level of contamination risk is not strictly identical, depending on its use.

⁶ The water in ports, which is always contaminated in some way, should never be used to wash fish (*Codex Alimentarius* Recommended International Code of Practice for Fresh Fish, CAC/RCP 09-1976).

Given these elements, AFSSA considers that seawater could be used for the above-mentioned purposes⁷, provided that the user can guarantee to the competent health authorities the quality of the clean seawater used, on the basis of a dossier containing:

- A preliminary study of the composition of raw seawater (including water in which drilling takes place) at the pumping point, and its possible variations.
- A risk assessment for the degradation of water quality.
- A study of the vulnerability of the resource and the protective measures to be implemented.
- The rationale for treatment products and processes to be used.
- Description of facilities for water production and distribution.
- Description of the procedures for monitoring water quality (own checks).

Since, pragmatically, it seems difficult to ensure continuous monitoring of the resource if it is vulnerable, AFSSA considers that **seawater should be subject to a treatment adapted to the quality of the resource, consisting of the following three steps:**

- A retention step for particles and colloids, in order to obtain a turbidity <0.5 FNU after treatment, ensuring the efficacy of the treatment (e.g., clarification),
 - An adsorption step, designed to remove chemical contaminants (e.g., activated carbon),
 - A disinfection step, to eliminate microbiological contaminants (e.g., UV),
- these steps would ultimately ensure the health and safety of consumers of fishery products.

3. The phycotoxin risk

Considering that no treatment is currently available to effectively retain or eliminate marine phycotoxins, AFSSA recommends suspending the pumping of seawater in cases where the REPHY⁸ alert threshold is exceeded at the phytoplankton monitoring point representative of the pumping point.

If upon further analysis the phytoplankton alert corresponds to a non-toxic species (or genera), pumping can resume.

4. Monitoring of the quality of the seawater used

AFSSA considers that the operator should establish a system for overall management of the quality of water produced, validated by the health authority. Possible deviations in resource quality will need to be monitored by appropriate analyses.

Moreover, AFSSA recommends that users of clean seawater establish a quality procedure, enabling them to identify through its use the hazards, potential risks, preventive measures and controls to apply, as well as general hygiene measures, cleaning and disinfection operations, and own checks to be implemented.

They should also ensure that storage conditions do not lead to deterioration in the quality of treated seawater.

In addition, site by site, the conditions for sampling and seawater discharge must be specified in order to minimise the impact on fishery products, on the environment, and on water treatment stations, in accordance with the regulations in force.

⁷ Water supply for fish and crustaceans tanks; washing and cooling of crustaceans and molluscs after cooking; handling and washing of unprocessed products such as fish fillets and slices; washing of whole, gutted, and beheaded fishery products; manufacture of ice for cooling and storage of fishery products, fresh or processed; cleaning of facilities and equipment

⁸ REPHY: French monitoring network for phytoplankton and phycotoxins, coordinated by Ifremer (French Research Institute for Exploitation of the Sea).

Special case of clean seawater use

Regarding the special case of the use of reconstituted seawater for fish and crustacean tanks, based on information provided by the DGAL, AFSSA does not have at this time sufficient data on the quality of the salts used (presence of chemical contaminants) to rule on the health and safety of the fishery products concerned (crustaceans). Regulatory clarification for this use would be desirable.

These are the elements that the French Food Safety Agency is able to provide to health authorities in response to the above-mentioned request.

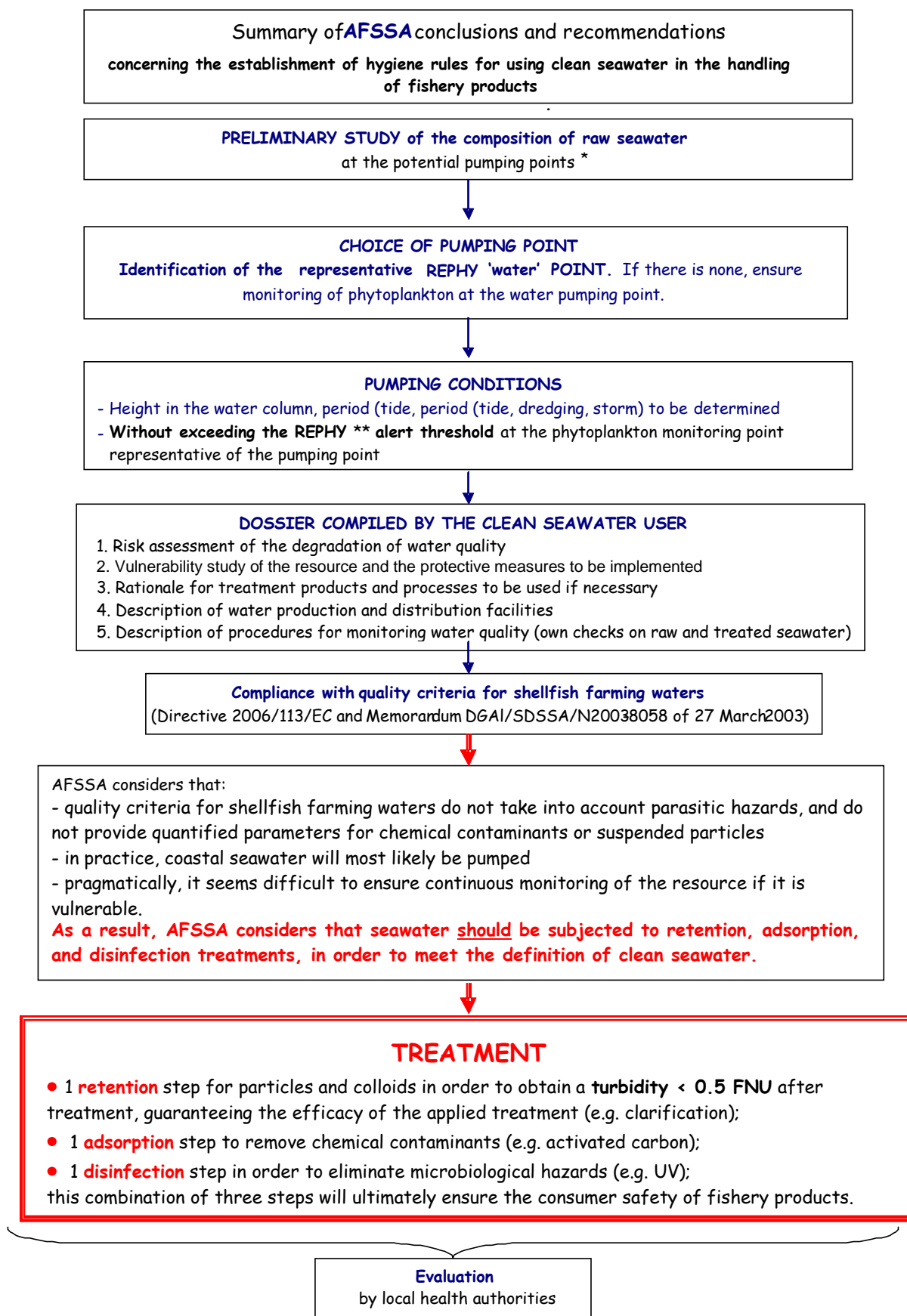
Moreover, AFSSA underlines the need:

- for an inventory of the uses of seawater by fish auctions and shore establishments;
- in applying the Hygiene Package, to develop guides to good hygiene practices and application of HACCP principles in the appropriate industries, taking into account this issue of the use of clean seawater.

In addition, as requested by the CES on Microbiology, it would be desirable to acquire knowledge about the behaviour of pathogenic microorganisms in the marine environment (*Vibrio*, *Listeria monocytogenes*, etc.) through environmental studies on seawater and fishery products.

The conclusions issued by the French Food Safety Agency are presented schematically in **Figure 3**.

Figure 3 :



*In the OSPAR Convention, the EAC (ecotoxicological assessment criteria) in sediments are indicative of a potential risk and may serve as a guide in choosing a pumping area, to avoid the risk of release of substances adsorbed on the particles.

**Unless after further analysis, the REPHY alert is proved to correspond to a non-toxic species or genera. REPHY is the French monitoring network for phytoplankton and phycotoxins

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10. KEYWORDS

Clean seawater, fishery products, microbiological contaminants, chemical contaminants, hygiene, phycotoxins, marine biotoxins.

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**Annex 1:
Assessment of Information Related to Microbiological Hazards of Interest in Fishery Products**

With regard to microbiological hazards, different types of microbiological contaminants may be present in seawater and consequently affect fishery products. Three main types of microbiological hazards are likely to affect fishery products:

- bacteria
- viruses
- parasites

1. Bacteria

Table 1. Factors limiting growth and heat resistance in pathogenic bacteria normally present in seafood (Group 1 - indigenous bacteria)

Pathogenic bacteria	Temperature (°C)		pH	a _w	NaCl (%)	Heat resistance
	minimum	optimum	minimum	minimum	maximum	
<i>C. botulinum</i>						
proteolytic type A, B, F	10	approximately 35	4.0–4.6	0.94	10	D ₁₂₁ from spores = 0.1 - 0.25 min
non-proteolytic type B, E, F,	3.3	approximately 30	5.0	0.97	3–5	D _{82.2} = 0.15–2.0 min in broth D ₈₀ = 4.5–10.5 min in products with high protein and fat content ⁶
<i>Vibrio</i> sp.	5–8	37	5.0			D ₇₁ = 0.3 min ¹
<i>V. cholerae</i>	5	37	6.0	0.97	<8	D ₅₅ = 0.24 min ²
<i>V. parahaemolyticus</i>	5	37	4.8	0.93	8–10	60°C for 5 min gave a decrease of 7 log ₁₀ for <i>V. parahaemolyticus</i>
<i>V. vulnificus</i>	8	37	5.0	0.94	5	
<i>Aeromonas</i> sp.	0–4	20–35	4.0		4–5	D ₅₅ = 0.17 min ⁵
<i>Plesiomonas</i> sp.	8	37	4.0		4–5	60°C/30 min. no survival ⁷
<i>Listeria monocytogenes</i>	1	30–37	5.0	0.92 ⁴	10	D ₆₀ = 2.4-16.7 min in meat products ³ D ₆₀ = 1.95–4.48 min in fish (Figure 3.3).

According to Doyle (1989), Buckle (1989), Farber (1986) and Varnam and Evans (1991)

¹ Schultz et al. (1984), ² Delmore and Crisley (1979), ³ Farber and Peterkin (1991), ⁴ Nolan et al. (1992), ⁵ Condon et al. (1992), ⁶ Conner et al. (1989), ⁷ Miller and Koburger (1986).

Table 2. Factors limiting growth and heat resistance in bacteria from the animal/human reservoir (Group 2 - non-indigenous bacteria)

Pathogenic bacteria	Temperature (°C)			pH	NaCl (%)	a _w	Heat resistance
	minimum	optimum	maximum	minimum	maximum	minimum	
<i>Salmonella</i>	5	37	45–47	4.0	4–5	0.94	D ₆₀ = 0.2–6.5 min
<i>Shigella</i>	7–10	37	44–46	5.5	4–5		60°C/5 min
<i>E. coli</i>	5–7	37	44–48	4.4	6	0.95	D ₆₀ = 0.1 min D ₅₅ = 5 min
<i>Staphylococcus aureus</i>	7	37	48	4.0	10–15	0.83	D ₆₀ = 0.43-7.9 min
<i>Staphylococcus aureus</i> toxin production	15	40–45	46	approx. 5.0	10	0.86	High stability of the toxin to heat

According to Doyle (1989), Buckle (1989), Varnam and Evans (1991) and Farber (1986)

Salmonella

The bibliographic data are in agreement on the following point: *Salmonella* does not survive long in seawater. The sensitivity of *Salmonella* to various factors is reported in Table 2. In addition, if a new pumping area is involved, the absence of *Salmonella* should be checked at the outset. (For reference purposes, *Salmonella* are not screened for in the water distribution system, or in mineral waters, but only in bathing water).

Listeria monocytogenes

Like *Salmonella*, *Listeria monocytogenes* has poor resistance to seawater salinity. The sensitivity of *L. monocytogenes* to various factors is reported in Table 1. However, this bacterium can be detected in coastal areas: as an example, a prevalence of 3.1% has been observed in samples of seawater at different sites in the coastal region of Agadir, Morocco (El Marrakchi *et al*, 2005). A temporary presence cannot therefore be excluded. An inventory is necessary, but a specific criterion for *L. monocytogenes* does not seem justified on the basis of current knowledge.

Staphylococcus aureus

The sensitivity of *Staphylococcus aureus* to various factors is reported in Table 2. This bacterium is resistant to high salt concentrations; this property may warrant special attention. In reality, the presence of *S. aureus* in food results in almost all cases from human infection (sick individuals or healthy carriers; presence on the skin and/or respiratory tract). *S. aureus* should therefore be screened for afterwards, notably at the end of the process, but there is no justification for retaining this criterion for seawater.

Vibrio

In seawater, vibrios are not a contaminating flora, but a natural, native flora; the question is whether any of them are pathogenic.

In its Opinion of December 1999⁹, AFSSA noted that the only potentially pathogenic foodborne vibrios in fishery products are *Vibrio cholerae* O1 and O139 and certain *Vibrio parahaemolyticus*. In its opinion of June 2003, AFSSA considered that for *V. parahaemolyticus*, only strains of *V. parahaemolyticus* possessing the genes encoding the hemolysins TDH and/or TRH are pathogenic.

With respect to *V. parahaemolyticus*, the implementation of analyses is currently very onerous for detection, enumeration (by the most probable number [MPN] method) and characterisation (by molecular methods). The implementation of a reliable and specific PCR method would be required to make proposals for *Vibrio* criteria.

For reference, vibrios are not currently included in the microbiological criteria of the EU regulation, but a recital of Regulation 2073/2005 mentions *V. parahaemolyticus*.

In conclusion, some methodological problems concerning vibrios remain to be resolved. Moreover, it is necessary to accumulate data by conducting studies.

The sensitivity of *V. parahaemolyticus* to various factors is reported in Table 1.

Indicators of contamination (indicators of pollution, and treatment efficacy)

- coliforms

Their lack of specificity limits the usefulness of screening for them. Indeed, many bacterial species belonging to the coliform group are part of the natural soil and aquaculture microflora.

- indicators of faecal contamination
 - *Escherichia coli*

This species has good specificity and a precise taxonomic definition. It is the indispensable indicator of faecal contamination. It is particularly interesting, in the context of this issue, to make the association with the shellfish industry.

The sensitivity of *E. coli* to various factors is reported in Table 2.

- Faecal enterococci (*Enterococcus faecalis*, *faecium*)

They are more specific than faecal streptococci (faecal streptococci and enterococci survive better than others in a marine environment).

⁹ Opinion No. 1999 SA-0013 of 2 December 1999 on *V. cholerae*, *V. vulnificus* and *V. parahaemolyticus*;
Opinion No. 2003 SA-0039 of 25 June 2003 on the revision of the Ministerial Order of 21 December 1979 (p 19-22).

2. Viruses

Viral hazard and fishery products

The risk of viral transmission to humans through food and/or water consumption is, in the current state of knowledge, limited exclusively to viruses belonging to the "faecal peril": Hepatitis A virus (HAV), Hepatitis E virus (HEV), Caliciviruses (Norovirus and Sapovirus), Rotavirus, Astrovirus, Adenovirus subtypes 40 and 41, Reoviruses, Enteroviruses and Parechovirus. These viral agents are spread by inter-human transmission; infection occurs through ingestion of contaminated food or water and, in this context, they are responsible for occasionally widespread outbreaks.

Because of their natural resistance, all of them can persist in the external environment and especially in seawater. On the other hand, viruses are unable to multiply in the external environment. The viral load of seawater will consequently be directly related to the quantity of virus released by humans (or animals) in the external environment. The aspect of faecal-oral transmission common to all these viruses involves the possibility of their waterborne transmission to food products with which they come in contact.

A review of recent literature and health alerts did not demonstrate a direct link of transmission of these viruses by fishery products; only shellfish are a very frequently implicated vector. The risk of viral transmission to humans by shellfish is directly related to their filtering and concentrating role. As shellfish are excluded from the scope of this request, the remainder of the document is therefore not applicable to the use of seawater for shellfish.

Viral contamination of seawater

The proximity of wastewater discharges resulting from human activities is the main source of seawater contamination by these 'enteric' viruses.

Environmental compartments where the transmitted viruses can be retained and persist, thus constituting a potential secondary viral source, are the soil, groundwater, surface water and sediment. The soil generally appears to provide an effective barrier to the transfer of viruses to the water table; in addition, the reduction measured in the saturated area (water table) is significant. In contrast, for surface water, the re-suspension of sediment contributes to a re-contamination of marine and environmental bodies of water. The risk associated with contaminated surface water also seems more significant, depending on various factors (mainly climatic and anthropogenic) which may be the source of its contamination.

An increase in temperature decreases the survival of viruses in seawater. A 90% reduction in poliovirus and parvovirus was obtained in 3 days at 28°C and 10 days at 6°C (Wait & Sobsey, 2001). (Wetz *et al*, 2004) found that an increase from 22° to 30°C in the temperature of filtered seawater leads to a reduction in poliovirus infectivity and RNA, which is faster at 22°C in unfiltered seawater; i.e., in the presence of bacterial flora.

In addition, the adsorption of viruses on particles favours the persistence of infectivity. In the absence of particles, infectious enteroviruses and rotaviruses disappear within 9 days, whereas they persist for 19 days in their presence (Rao *et al*, 1984a). In estuaries, enteroviruses are mainly present on suspended particles (LaBelle & Gerba, 1979). An estimate of viral distribution isolated from Galveston Bay (USA), indicated that most viruses are associated with suspended particles with a size <3 µm and with flaky sediment. (Schernewski & Julich, 2001a) assessed the spatial impact of the release of waste products in the Oder estuary, and suggested that only viruses associated with particles are able to remain infectious and to be disseminated over a distance.

Regulation and monitoring of viral presence/seawater pumping areas

Regulation (EC) No. 2073/2005 states in its second recital that foodstuffs should not contain microorganisms or their toxins or metabolites in quantities that present an unacceptable risk for human health. In this regulation, the following are defined under the term microorganisms: bacteria, viruses, yeasts, moulds, algae, parasitic protozoa, and microscopic parasitic helminths, as well as their toxins and metabolites. Viruses are therefore included in this context. However, no microbiologic regulatory criterion has been established for viruses in any type of food matrix. This is particularly due to a lack of sufficiently reliable analytic methods. The regulation specifies in its 27th

recital that it is necessary in particular to establish criteria for pathogenic viruses in live bivalve molluscs, when the analytical methods are developed sufficiently.

In addition, Regulation (EC) No.2073/2005 states in its 12th recital that on 30 and 31 January 2002, the Scientific Committee on Veterinary Measures Relating to Public Health (SCVPH) issued an opinion on Norwalk-like viruses (or noroviruses). In its opinion, the committee concluded that "conventional faecal indicators are unreliable for demonstrating the presence or absence of Norwalk-like viruses, and that the reliance on faecal bacterial indicator removal for determining shellfish purification times is unsafe practice." The committee also recommended using *E. coli* rather than faecal coliforms to indicate faecal contamination in shellfish harvesting areas, when applying bacterial indicators. Therefore, it is the *E. coli* indicator which is recommended for shelled and shucked products of cooked crustaceans and molluscs, and which enables the quality of the analysed batches to be tested. The development of analytical methods would appear to be essential before establishing criteria applicable to pathogenic viruses in live bivalve molluscs. For this, a working group was set up to validate a horizontal method for detection of noroviruses and HAV in foods, using real time RT-PCR (CEN/TC275/WG6/TAG4 Group).

For fishery products in the strict sense, the viral risk associated with seawater must be distinguished from that of transmission by humans during the processing of these products. Indeed, one of the modes of transmission most frequently identified for these viruses is the contamination of food by the dirty hands of a sick operator. This essential element must be taken into account in the evaluation of the different uses of seawater and its cleanliness levels (in the processing room).

It seems essential that the water used for worker hygiene, particularly hand washing in the work room, be drinking water, and that the seawater used in these same workshops be of equal quality, and therefore treated.

3. Parasites

Overview of parasites of interest

The detection of parasites in water intended for human consumption in France is not systematic; it is considered in the context of self-monitoring, and as part of the quality of raw water during the application for approval of the treatment facility.

The various regulations (Order of 11 January 2007, Directive 2006/7/EC, Directive 2006/113/EC) apply respectively to water intended for human consumption, to the management of bathing water quality, or to the quality required of shellfish farming waters, and do not establish quality limits specifically related to parasites. Parasites (especially protozoa) constitute a risk for water contamination, and several waterborne outbreaks of parasitic origin have been reported. Among the parasites most often implicated, *Cryptosporidium spp.* and *Giardia* are mainly found, possibly because detection techniques applicable specifically to water exist principally for these two microorganisms (NF T 90-455, July 2001). The detection of these parasites is listed as an optional analysis in the Order of 24 January 2005 relative to approval conditions for laboratories for conducting sampling and health monitoring of water (J.O.[Official Journal], 22 February 2005).

Among the parasites, **protozoa** represent the major risk, especially because of their ubiquitous nature and because they have an environmental dissemination phase in their cycle. These parasites are excreted by different hosts (domestic, synanthropic and wild mammals; humans) as cysts or oocysts, whose main characteristic is that they are particularly resistant to environmental temperature and humidity conditions. In the environment, soil leaching is an important factor in dissemination, a source of contamination of natural resources. **Diffusion in seawater is therefore possible.** The great resistance of protozoa to current conventional disinfection methods (Koriche *et al.*, 1990) makes the assessment of these health risks indispensable, in order to better identify the actual exposure of the population. The main parasites that can cause water contamination are described below:

Cryptosporidium is a parasite excreted in large quantities by infected humans or animals (10^7 to 10^9 per gram of faeces for a calf). The contamination level of natural waters depends on many factors, including soil leaching, and consequently rainfall. Average contamination levels also depend on the nature of the resources studied (surface water, groundwater in karst areas), with

levels often high, especially in farming areas. Many outbreaks related to contamination of the water supply have been reported worldwide (LeChevallier, 1991, 1995).

Giardia: most information on risk factors for giardiasis has been obtained from investigations of reported outbreaks. Water is a frequently identified mode of transmission, and the consumption of tap water is recognised as a risk factor for giardiasis (Moorehead, 1990; Levy, 1998). This disease represents, along with cryptosporidiosis, **one of the most important water-related public health problems in developed nations** (Thurman, 1998). For *Giardia*, the same parasite sources as those mentioned for *Cryptosporidium* oocysts can be incriminated. *Giardia* cysts survive for a significant length of time. In human or cattle faeces, it varies from 15 to 30 days (maximum 74 days). In surface water, the survival of cysts varies from 28 to 56 days, depending on temperature conditions. The cysts can remain viable at 4°C for 90 days, and for 66 days between 12 and 22°C. In sampled surface waters, the percentage of viable cysts recovered varies from 3.5% to 18%. Other eukaryotic microorganisms classified as emerging may be responsible for water contamination. **Microsporidia** represent a risk, especially for immunocompromised patients. They constitute a very large group of parasitological micro-fungi, which includes over 100 genera containing more than 1000 different species, of which only a low percentage (11 species) is responsible for infection in patients. While numerous species infest the animal world, four species are mainly responsible for human disease: *Enterocytozoon bienersi*, *Encephalitozoon intestinalis*, *E. hellem* and *E. cuniculi*. Malabsorption with chronic diarrhoea is the most common clinical manifestation of intestinal microsporidiosis. A parasitic opportunist, microsporidiosis is mainly observed in profoundly immunocompromised AIDS patients. In the United States, *E. bienersi* and *E. intestinalis* have also been found in surface water and different types of effluent, confirming parasitic circulation in the natural environment (Dowd, 1998). Moreover, the involvement of tap water in a microsporidiosis outbreak was strongly suspected in Lyon in 1995 (Cotte, 1999). As with *Cryptosporidium* and *Giardia*, contamination associated with bathing water has been described, but French regulations do not impose parasitological criteria among the monitoring criteria. Microsporidia that are pathogenic to humans, however, are clearly present in fresh surface water and swimming pools in France (Coupe *et al*, 2006, Fournier *et al*, 2000, 2002) and in freshwater bivalves in Ireland (Graczyk *et al*, 2004). **These organisms could therefore be found in the coastal sea.**

The Apicomplexa *Toxoplasma gondii* is a newly recognised waterborne pathogen. Contamination is due to oocysts excreted by cats at the waning of an infection. These parasitic forms are non-sporulated when released into the environment (i.e. not directly infective), with sporulation occurring within a few days (depending on the temperature and humidity of the environment), generating infectious oocysts which are extremely resistant in the environment. Oocysts can retain their infectivity in the soil for 18 months at various temperatures (Frenkel, 1975). Survival conditions in water have been evaluated experimentally for temperatures observable under natural conditions. Non-sporulated oocysts do not lose their infectivity after storage at 4°C for 6 to 11 weeks (Lindsay, 2002). Sporulation is possible in seawater at 24°C (artificial seawater at 15 and 32 g/L) (Lindsay, 2003). Sporulated oocysts can survive and remain infectious in water at room temperature for 15 months (Hutchison, 1967), at 4°C for at least 54 months (Dubey, 1998a), without loss of infectivity for 18 months (Dubey, 1998), and for 6 months in seawater (15 g/L) at room temperature or at 4°C (Lindsay, 2003). Sporulated oocysts retain their infectivity after freezing for 28 days at constant -21°C (Frenkel, 1973) and for 106 days at temperatures of -5 and -10°C. On the other hand, they lose their infectivity at high temperatures (in 2 days at 45°C, 2 hours at 50°C, and 1 minute at 60°C, [Dubey, 1998]). The largest waterborne outbreak linked to this parasite occurred in 1995, in the metropolitan region of Victoria (British Columbia), with 110 infections identified among the civilian population. This outbreak of toxoplasmosis is the largest ever observed, and the first to have been associated with municipal drinking water (Aramini, 1998, Bowie, 1997). Other outbreaks have since been reported (Bahia-Oliveira, 2003; de Moura, 2006). The only detection method proposed for *T. gondii* required inoculation in mice with pellets of water (after filtration/elution), with a response time of about 7 weeks (Isaac-Renton, 1998). Since then, other detection techniques have been developed based on molecular methods (Kourenti, 2003; Villena, 2004), or detection using specific monoclonal antibodies (Dumètre, 2005). Detection of toxoplasma DNA in water intended for human consumption has been reported in France (Villena, 2004).

Among **other parasites spread by faecal-oral transmission**, *Cyclospora cayatanensis* is considered primarily a waterborne pathogen, since it is largely transmitted through water contaminated with faeces. *Cyclospora* oocysts are resistant to chlorination (Rabold, 1994) but are destroyed by boiling. While this mode of transmission remains high in places like Peru, Nepal, Java,

Guatemala, and Haiti, where the parasite is endemic, many foodborne outbreaks have been reported in recent years in Canada and the United States. Outside the United States and Canada, most reported cases of cyclosporiasis in Europe and Australia have been associated with travel to countries where the disease is endemic (tropical countries). Diarrhoea and weight loss are the main symptoms of this parasitosis, rare in France. **The risk of seawater contamination seems very low.**

It is important to note that **in most waterborne parasitic outbreaks, sanitary water inspection was compliant with regulations.** In outbreaks related to seafood, parasites do not appear to be frequently involved, probably because these agents are not screened for as a potential source of infection. In France, these parasites are not systematically screened for in the stools of subjects presenting digestive problems with diarrhoea, or in seafood, drinking water, or bathing water.

Elements concerning the evaluation of parasitic risk

Health risk assessment related to waterborne protozoa is poorly understood, mainly due to the **cumbersome detection techniques** proposed, compared to conventional methods of detection for other microorganisms in water; it is currently only proposed for the two main acknowledged pathogens (*Cryptosporidium* and *Giardia*) in cases of suspected water contamination (high turbidity, for example). Another reason for the lack of knowledge about the waterborne health risk related to other parasitic protozoa is the **lack of specific methods for their detection in the environment.** Since river water will be found in seawater, and other bodies of water (lakes, etc.) can run off into the sea, the presence of these parasites in seawater cannot be excluded. Many parasites can be ingested by fish or shellfish, bivalves playing the role of concentrating organisms. The presence of *Cryptosporidium*, *Giardia* and microsporidia has additionally already been demonstrated in mussels and oysters in the USA (Fayer, 1998; Graczyk, 2003) and Europe (Li *et al*, 2006, Pereira *et al*, 2006; Melo *et al*, 2006).

Seawater salinity could affect the viability of these parasites. A salinity of 45 g/L (versus 5 g/L) accelerates the loss of viability of *Cryptosporidium* oocysts stored either at 4°C (x 2.0) or at 30°C (x 1.2). However, other data indicate that the oocysts of *C. parvum* can survive in seawater at 6-8°C for 1 year (Erickson and Ortega, 2006).

In addition, when using seawater for ice production, the destruction or inactivation of the above-mentioned parasites might be assumed. However, caution should be maintained, given that *C. parvum* can survive for a long time in ice made from drinking water. In milk or fruit juice, *C. parvum* remains alive for over three weeks at -20°C. As for *Giardia*, it requires 84 days at 0-3°C to completely neutralise the infectivity of the cysts (see review: Erickson and Ortega, 2006). On the other hand, data concerning the survival of *Cryptosporidium parvum* in sorbet indicate that only a low proportion of oocysts survived 24 hours at -20°C (Deng and Cliver, 1999). Finally, *T. gondii* oocysts may remain viable after freezing for 28 days at constant -21°C (Frenkel, 1973; Smith, 1991), and without loss of infectivity for 106 days at -5°C and -10°C (Dubey, 1998). Thus, freezing may not be sufficient to kill all sporulated oocysts (Frenkel, 1973).

Disinfection procedures and their efficacy on parasites: UV ozone effect on parasites

Concerning *Cryptosporidium*, UV has significant inactivation potential with respect to this parasite; the efficacy of inactivation depends on the UV dose (expressed in mJ/cm²) actually received by the oocysts. For water treatment, a dose of 25-40 mJ/cm² would achieve a reduction of two to three log for a very low turbidity (AFSSA Report, 2002). Treating *Cryptosporidium* oocysts with 1 mg/L ozone for 5 min inactivates about 90% of parasites (Korich, 1990). Filtration, in particular nano-filtration, is the best technique for retention of *Cryptosporidium* oocysts. For *T. gondii* oocysts, there are no published data on the activity of UV and ozone (AFSSA Report, 2005).

Parasite detection

Detection of parasites in water involves a different approach to that proposed for the detection of bacteria and viruses. Detection methods exist for this and are already used (Matheson *et al*, 1998), but only the simultaneous detection of the protozoa most commonly involved in waterborne epidemics (***Cryptosporidium* and *Giardia***) has been standardised.

Thus, **there is a standardised method for detection and enumeration of *Cryptosporidium* oocysts and *Giardia* cysts in water** (NF 90-455 T, July 2001). It includes three successive steps: the parasites are concentrated by filtration through a 1 µm porosity cartridge; after elution and centrifugation, the pellet is recovered and the parasites concentrated by magnetic immunoseparation with magnetic beads on which specific antibodies for the two protozoa are

bound. Detection and enumeration of oocysts and cysts is then carried out by immunofluorescence, using specific antibodies coupled with fluorescein isothiocyanate and counting with a fluorescence microscope. Detection techniques by flow cytometry have also been developed. However, the total yield of the described method is low (about 30 to 50%), due to loss of parasites at each step (primarily during the first filtration step). To improve detection, it is necessary to filter large volumes (100 litres); but the clogging of cartridges, often observed in highly turbid water, further limits the method's possibilities.

There is no specific standardised detection method for other parasites. However, the various described methods generally always have a preliminary concentration step, particularly by filtration, such as that recommended for *Cryptosporidium* and *Giardia*. In the absence of specific monoclonal antibodies for other parasite species, coupled magnetic beads are not available, making it impossible to practice immunoseparation techniques. The parasites are then generally detected by PCR.

For *T. gondii*, the recent description of a method based on the use of monoclonal antibodies (Dumètre, 2005) could improve detection of this parasite in the environment; the two other reported methods for the time being are mouse inoculation (with delayed response time of 4 weeks) (Isaac-Renton, 1998; de Moura, 2006) or molecular biology (Villena, 2004).

Detection of *Cryptosporidium* and *Giardia* in water is not directly correlated with that of the bacteria principally observed during contamination of the system (*E. coli*, total coliforms and enterococci); however, the presence of sulfite-reducing bacteria, including sporulated forms, may trigger screening for the presence of these parasites. Generally, high turbidity indices are associated with the detection of *Cryptosporidium* and *Giardia* in water. The parallel increase in protozoa concentrations and turbidity during treatment of the water production facility confirm the usefulness of monitoring this parameter as an indication of proper operation of clarification facilities (Robert, 2006).

Annex 2:

**OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic,
Quality Status Report 2000**

The Convention for the Protection of the Marine Environment of the North-East Atlantic, 1992 (called the "OSPAR Convention"), is responsible for taking all possible measures to prevent and eliminate pollution, as well as taking necessary measures for the protection of the maritime area against the adverse effects of human activities, in order to safeguard human health and conserve marine ecosystems and, where feasible, restore marine areas which have been adversely affected. As such, the OSPAR Commission conducts various studies and research on the quality of the marine environment. In particular, it has collected data on background reference concentrations and has proposed ecotoxicological assessment criteria.

1. Background Reference Concentrations (BRCs)

These are values which reflect uncontaminated natural concentrations.

Inorganic contaminants

Ranges of background reference concentrations of metals in fine-grained marine sediments, in seawater and in the common mussel in the OSPAR zone.

	Sediments (metal/Al (x 10⁻⁴) ratio)	Seawater (ng L⁻¹)	Common mussel (mg kg⁻¹ fresh weight)
Cadmium	0.007 – 0.04	5 – 25	0.07 – 0.11
Mercury	0.0034- 0.0066	0.1 – 0.5	0.005 – 0.01
Lead	1.8 – 4	5 – 20	0.01 – 0.19
Copper	2.2 – 5.7	50 - 360	0.76 – 1.1

Organic contaminants

Organic contaminants are anthropogenic for the most part. These are chlorinated compounds from industry and agriculture, pesticides, PCBs, and in particular PAHs, whose sources for seawater contamination are the most diverse and the most frequent. They are all hydrophobic compounds with very low solubility in water, whose concentrations are very low in open sea but much higher along the coasts, especially in port areas, estuaries and around industrial plants. There is no longer seawater anywhere in the world exempt from trace organic contamination. These hydrophobic compounds, generally highly volatile, are transported by the major atmospheric currents. For PAHs, reference concentrations in the open sea are given in the following table.

Ranges of background reference concentrations of PAHs in surface water (ng/L).

	Northern North Sea	Central and Southern North Sea	North-East Atlantic
Benzo(a)pyrene	0.002 – 0.005	0.002 – 0.004	0.001
Fluoranthene	0.073 – 0.285	0.104 – 0.264	0.036 – 0.054
Benzo(b)fluoranthene	0.004 – 0.017	0.003 – 0.009	0.001 – 0.004
Pyrene	0.014 – 0.053	0.011 – 0.024	0.02 – 0.033

2. Ecotoxicological Assessment Criteria (EAC)

Ecotoxicological Assessment Criteria are defined as the concentration levels at which a concern is justified. These criteria aim to protect marine flora and fauna, and have no connotation for human health. The following tables show the EAC of some inorganic and organic contaminants.

Ecotoxicological assessment criteria applicable to metals.

	Seawater ($\mu\text{g L}^{-1}$)	Sediments (mg kg^{-1} dry weight)
Cadmium	0.01 – 0.1	0.1 – 1
Copper	0.005 – 0.05	5 – 50
Mercury	0.00 – 0.05	0.05 – 0.5
Lead	0.5 – 5	5 – 50
Zinc	0.5 – 5	50 – 500

Ecotoxicological assessment criteria applicable to PCBs, PAHs, TBT and some organochlorine pesticides.

	Water $\mu\text{g/l}$	Sediments mg/kg dry weight
DDE	nr	0.0005 – 0.005
Dieldrin	nr	0.0005 – 0.005
Lindane	0.0005 – 0.005	nr
Naphthalene	5 – 50	0.05 – 0.5
Phenanthrene	0.5 – 5	0.1 – 1
Anthracene	0.001 – 0.01	0.05 – 0.5
Fluoranthene	0.01 – 0.1	0.5 – 5
Pyrene	0.05 – 0.5	0.05 – 0.5
Benz(a)anthracene	nd	0.1 – 1
Chrysene	nd	0.1 – 1
Benzo(a)pyrene	0.01 – 0.1	0.1 – 1
7PCB	nr	0.001 – 0.01
TBT	0.00001 – 0.0001	0.000005 – 0.00005

nr: not part of the current monitoring program;

nd: no available data, or available data are insufficient;

7PCB = sum of congeners 28, 52, 101, 118, 138, 153, 180

The EAC in sediments are indicative of a potential risk. They could be used to guide the choice of most suitable pumping area for avoiding the risk of release of substances adsorbed on particles.

REFERENCE

OSPAR Commission. Quality Status Report 2000. London. 108 + vii pp.

http://www.ospar.org/content/content.asp?menu=00650830000000_000000_000000