The Director General

Maisons-Alfort, 15 May 2020

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the update of the risk assessment on the presence of cyanobacteria and their toxins in drinking water, recreational water and water intended for professional and recreational fishing activities

ANSES undertakes independent and pluralistic scientific expert assessments.

ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.

It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are published on its website. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 15 May 2020 shall prevail.

On 19 July 2016, ANSES received a formal request from the Directorate General for Health (DGS) to update the risk assessment on the presence of cyanobacteria and their toxins in drinking water (DW), and recreational water.

Previously, on 25 September 2015, ANSES had received a formal request from the Directorate General for Food (DGAL) and the DGS for scientific and technical support on a review of knowledge concerning the contamination of freshwater fish by cyanotoxins.

1. BACKGROUND AND PURPOSE OF THE REQUEST

The request to ANSES followed firstly, the presence of toxigenic cyanobacteria in resources used for DW production and secondly, recurrent observations of cyanobacterial blooms in water bodies, which had led to a temporary ban on recreational activities (bathing, water sports), as well as on professional and recreational fishing due to the risk of fish being contaminated by cyanotoxins. The DGS's request for an update referred to a previous request to the French Food Safety Agency (AFSSA) in 2001 and to the French Agency for Environmental and Occupational Health Safety (AFSSET) in 2004, on the risks associated with the presence of cyanobacteria and cyanotoxins in DW and in recreational water used for bathing or for other recreational activities. A joint report by these two agencies had then been published (AFSSA – AFSSET, 2006). The need for the update requested by the DGS followed developments in scientific knowledge on cyanobacteria and the toxins they can produce, since publication of the 2006 report, and the acquisition of numerous analytical results from the resources used to produce DW, from DW itself and from recreational water.

Cyanobacteria are bacterial micro-organisms that thrive in terrestrial and aquatic environments, whether brackish, marine or fresh water. In favourable environmental conditions (i.e. with regard to temperature and nutrients), they can proliferate rapidly in just a few days on a massive scale; this is known as a bloom. In some cases, these blooms lead to a change in the colour of the water, a foul odour and/or an accumulation of cyanobacteria on the water surface. Cyanobacteria are being observed more and more frequently, on all continents, explaining the growing international concern about the associated ecological, health and economic consequences.

Biologically, cyanobacteria are Gram-negative photosynthetic bacteria with pigmentation ranging from blue-green to red. They are still sometimes referred to as blue-green algae, despite this being biologically incorrect. Indeed, from a systematic point of view, these micro-organisms belong to the kingdom of Eubacteria. However, for a long time they were classified in the plant kingdom, based on their photosynthetic activity, a characteristic previously assumed in aquatic environments to be specific to algae. The cell structure, in particular the absence of a nucleus and intracellular organelles, is nevertheless characteristic of the prokaryotic cells of bacteria. Like algae, most cyanobacteria in inland waters perform oxygenic photosynthesis coupled with CO_2 fixation, with water as the electron donor. They contain chlorophyll-a. But the presence of other photosynthetic pigments, characteristic phycobiliproteins, is the current basis for their identification.

In the aquatic environment, cyanobacteria are divided into two groups according to their way of life: planktonic and benthic. Planktonic cyanobacteria remain suspended in the water column due to intracellular gas vesicles that give them buoyancy. This characteristic explains their ability to accumulate on the water surface. Benthic cyanobacteria, on the other hand, grow on the bottom of water courses, on mineral substrates (boulders, pebbles, sand and sediment, for example) and even on the surface of macrophytes (aquatic plants).

In temperate zones, cyanobacterial blooms occur more often in the summer and early autumn, when there is abundant sunshine and water temperatures are above 20°C. However, they can sometimes be observed as early as the spring. In some rare cases, longer lasting blooms are seen throughout the year, and even specifically in winter. In tropical and subtropical climates, given the right conditions for their growth, blooms can be observed all year round.

Some species of cyanobacteria produce toxins called cyanotoxins that have a wide variety of chemical structures. The same species of cyanobacteria can produce different toxins and the same toxin can be produced by different species of cyanobacteria. Within the same species, some strains have the genes for synthesising toxin production while others do not. The best-known toxins are microcystins (MCs), cylindrospermopsins (CYNs), nodularins (NODs), anatoxins (ATXs), saxitoxins (STXs) and their derivatives, as well as lyngbyatoxins and aplysiatoxins. Each toxin can itself have many variants, resulting from structural variations. For example, more than 250 variants are now known in the microcystin family.

Because cyanotoxins remain predominantly in cyanobacterial cells until the lysis[1], the cyanobacterial species potentially producing toxins are considered in this opinion to be a hazard in DW and recreational water. Indeed, potentially toxin-producing cyanobacteria can lead to human exposure to cyanotoxins. The work discussed in this opinion deals only with freshwater cyanobacteria. Marine cyanobacteria and their associated toxins were not included in the scope of the expert appraisal in view of the extent of the work required for the update requested by the DGS and the DGAL.

The DGS therefore asked the Agency to carry out a scientific and technical expert appraisal to update:

- the list of toxigenic cyanobacteria species likely to be identified in freshwater (resources intended for DW production or water bodies intended for bathing, recreational activities and/or fishing) in metropolitan France and in the overseas *départements*;
- quality limit values for the concentration of cyanobacteria and/or their toxins in water intended for drinking and bathing.

[1] Lysis: rupture or destruction of the plasma membrane

ANSES assigned the reference number 2016-SA-0165 to these requests.

The DGAL and the DGS also asked ANSES to shed light on the contamination of freshwater fish, in particular on the following points:

- the state of knowledge concerning the toxicokinetics of the various cyanotoxins in freshwater fish likely to be consumed by humans, in particular:
  - their bioaccumulative capacity, specifying where appropriate the distribution of toxins in the various organs/tissues;
  - the possible link between toxin concentrations measured in water and/or cyanobacteria and those measured in fish;
  - the elimination rate of toxins;
- the different analytical methods to be recommended for cyanotoxins in fish;
- insights on lifting bans on fish consumption following cyanobacterial bloom episodes (e.g. levels falling back below a threshold of cell concentration or cyanobacteria biomass in the water).

Secondly, and in coordination with Request No. 2016-SA-0165, ANSES was asked to:

- propose health thresholds in fish on the basis of an update of the available toxicological knowledge on the various cyanotoxins likely to cause acute or chronic toxic effects in humans;
- investigate the possibility of correlating the updated management thresholds for bathing health risks with the risk associated with fish consumption;
- propose monitoring protocols to be implemented specifically to cover the food risk in addition to the monitoring of bathing water and independently of the current cyanobacteria thresholds.

ANSES assigned two numbers to these requests:

- Request No. 2015-SA-0206 concerning the first part of the request relating to the review of knowledge; this was addressed in the form of scientific and technical support (AST) involving a systematic review of the literature, with the work being finalised on 12 July 2016 (ANSES, 2016a);
- Request No. 2015-SA-0207, for the second part of the request, concerning the proposed maximum concentrations of cyanotoxins in freshwater fish and the monitoring protocols.

This current opinion brings together the conclusions and recommendations of the expert appraisal work relating to Request Nos. 2015-SA-0207 and 2016-SA-0165. The specific work on cyanotoxins drew on the opinions relating to Request Nos. 2016-SA-0297, 2016-SA-0298 and 2016-SA-0299 on the development of toxicity reference values for MC-LR, CYN and STX respectively.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General requirements of Competence for Expert Appraisals (May 2003)".

It falls within the sphere of competence of the Expert Committees (CESs) on "Water" and on "Assessment of physico-chemical risks in food" (ERCA). ANSES entrusted examination of Request Nos 2016-SA-0165 and 2015-SA-0207 to the "Cyanobacteria" Working Group (WG), which was set up on 6 January 2017 following a call for applications.

Two rapporteurs within the "Cyanobacteria" WG were appointed to carry out an initial expert appraisal of the work relating to Request No. 2015-SA-0207, supplemented by an in-house expert appraisal within the Food Risk Assessment Unit (UERALIM) of the Risk Assessment Department (DER) for the systematic review of the literature and the statistical processing of data.

The methodological and scientific aspects of the work of the "Cyanobacteria" WG relating to Request No. 2015-SA-0207 were regularly submitted to the CES ERCA at plenary sessions between 11 April 2019 and 23 October 2019. The document produced by the WG takes into account the comments and additional information provided by the members of the CES and by the reviewer appointed to carry out

a critical review of the document. The work was adopted by the CES ERCA at its meeting on 23 October 2019.

The methodological and scientific aspects of the work of the "Cyanobacteria" WG relating to Request No. 2016-SA-0165 were regularly presented to the CES on "Water" between 10 October 2017 and 4 February 2020. The report and the summary take into account the comments and additional information provided by the members of the CES and by the reviewers appointed to carry out a critical review of the report. The report was adopted by the CES on “Water” at its meeting on 7 January 2020; the summary was adopted on 4 February 2020.

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.

The experts’ declarations of interests are made public via the website of the Ministry of Solidarity and Health (https://dpi.sante.gouv.fr).

3. ANALYSIS AND CONCLUSIONS OF THE "CYANOBACTERIA" WG

Since the Agency's previous work on cyanobacteria, the presence of cyanobacterial blooms in surface water in metropolitan France and the French overseas territories has been broadly confirmed. The great diversity in these organisms and in their mode of development leads to widely differing situations, mainly according to the types of water bodies concerned and the type of cyanobacteria encountered (planktonic or benthic). Blooms are regularly accompanied by the production of cyanotoxins. In surface freshwater, the most widely screened for are microcystins (MCs), including many variants. Nevertheless, episodes of contamination by other cyanotoxins, in particular anatoxins (ATXs) and saxitoxins (STXs), have been reported in metropolitan France in recent years.

Massive cyanobacterial blooms can have ecological, health and economic consequences:

- ecological, because they can affect the health of ecosystems. High densities of cyanobacteria can alter the physico-chemical and ecological functioning of ecosystems. For example, the decomposition by aerobic chemo-organotrophic bacteria (often referred to as heterotrophs) of organic matter produced by cyanobacteria can remove oxygen from the water column, resulting in mass mortality of fish and invertebrates;
- health, through the production of cyanotoxins that can pose a health risk to humans and animals coming into contact with and/or consuming contaminated water. Cases of animal mortality, mainly concerning dogs, but sometimes also livestock or wildlife, have been recorded in recent years following exposure to blooms of cyanobacteria (mainly benthic); These events were correlated with the presence of ATX-producing cyanobacteria;
- economic, because the repulsive appearance of the water bodies due to the change in water colour, the possible accumulation of high densities of cyanobacteria on the surface and/or on the banks, and the unpleasant odours can lead to limitations on aquatic uses such as bathing, water sports or fishing. Cyanobacterial blooms can therefore have direct negative effects on the tourist industry along the shores of hydrosystems , and these may then be exacerbated by health restrictions on recreational uses. In resources used for DW production, cyanobacterial blooms and the production of cyanotoxins and/or unpleasant smelling compounds (other cyanobacterial metabolites) increase the cost of producing DW, for example by increasing the cost of water treatment or through the need to adjust treatment systems to deal with this problem.

In June 2003, the DGS drew up recommendations for quality monitoring and management of cyanobacteria in bathing water. The recommendations made by ANSES in 2006 were used to propose methods for implementing quality monitoring of DW and bathing water. The Ministerial Order of 11 January 2007 on the quality references and limits for raw water and drinking water mentioned in Articles R. 1321-2, R. 1321-3, R. 1321-7 and R. 1321-38 of the French Public Health Code advocates a quality limit of 1 µg.L^{-1} for total microcystins in drinking water. Monitoring results have been collected in the SISE-Eaux and SISE-Baignade databases. After analysing these data, the experts of the "Cyanobacteria" WG found that the lack of uniformity in the protocols used for quality monitoring, data storage and processing of analysis results prevent them from being fully utilised to conduct a health risk assessment at national level. In addition, these databases do not contain information on benthic

cyanobacterial blooms. Nevertheless, this data collection confirmed that the phenomenon of cyanobacterial blooms concerns the entire country (metropolitan France and the overseas territories, although there are few data available in the databases for the latter) and that the number of sites subject to cyanobacterial blooms seems to be increasing over time. The intensification of these phenomena can be explained locally by greater anthropic pressure in the catchment areas of the water bodies concerned, and the silting up and/or low renewal rate of certain water bodies.

In addition, the impact of climate change on cyanobacterial blooms is currently being debated by the scientific community. The global increase in temperature, combined with changes in rainfall patterns – reflected in the multiplication of periods of severe drought alternating with episodes of storms and violent rainfall – are causing changes in the hydrology of catchment areas (e.g. increasingly long-lasting and severe low river water levels) and in the physical functioning of water bodies (e.g. longer stratification periods of lakes). These changes appear to promote cyanobacterial blooms. However, the multiple interactions between all these factors and processes are still largely unknown. It is therefore very difficult to predict what their impact on blooms of potentially toxic cyanobacteria will actually be.

In temperate climates, cyanobacterial blooms occur more often in the summer, when there is abundant sunshine and water temperatures are above 20°C, but also sometimes in spring. These blooms can continue into the autumn. In some rare cases, longer lasting blooms are seen throughout the year, and even specifically in winter. In tropical and subtropical climates, given the right conditions for their growth, blooms can be observed all year round.

Planktonic cyanobacterial blooms occur mainly in eutrophic stagnant water (water bodies and very slow-flowing rivers). Indeed, to support biomass production, they require high concentrations of phosphorus (P) and nitrogen (N), which can be supplied directly or indirectly from many different sources (for example, livestock manure, compost, sewage sludge, fertilisers applied to agricultural soil, insufficiently treated wastewater discharges, and leaching from soil during heavy rainfall). **Reducing phosphorus and nitrogen inputs to surface waters is currently still the only sustainable way to restore and/or protect these ecosystems** from planktonic cyanobacterial blooms.

Benthic cyanobacterial blooms are most often found in shallow running water (small rivers and some large rivers) with a trophic status ranging from oligotrophic[2] to eutrophic[3]. Current knowledge of these blooms is far more limited than for planktonic cyanobacteria. However, it seems that benthic cyanobacterial biofilms mainly develop during periods of prolonged low water levels, in areas with depths of less than 1 m and with a current of around 0.2 to 1 $m.s^{-1}$. The detachment of these biofilms, their transport and then their accumulation on the banks occur as a result of various processes that are still poorly understood.

In view of the new scientific knowledge generated since the Agency's previous work (2006), the experts have made a series of recommendations designed to improve how the hazard associated with the presence of cyanobacteria in France (metropolitan and overseas territories) is taken into account, in order to limit the exposure of populations to cyanotoxins. The proposed recommendations aim firstly to optimise the management of water resources used for DW production and of DW production plants and secondly, to optimise the management of aquatic environments used for bathing and water sports. Points to assist with the management of contamination situations in water bodies used for professional or recreational fishing have also been provided.

The proposed measures relate mainly to:

- the taxonomy of toxigenic cyanobacteria;
- the toxicology of cyanotoxins;
- detection and quantification of cyanobacteria;

[2] An oligotrophic environment is one that is poor in nutrients. In an oligotrophic environment, the water has a low mineral content, is well oxygenated and very clear.

[3] A distinction should be made between natural eutrophication, which occurs on a geological time scale, and anthropogenic eutrophication, which corresponds to an excessive and rapid input of nutrients into the water, leading to a massive proliferation of primary producers, including cyanobacteria, oxygen depletion and an imbalance in the ecosystem.

- detection and quantification of cyanotoxins;
- means of preventing and controlling cyanobacterial blooms;
- considering the risks associated with the fishing and consumption of freshwater fish;
- the strategy for surveillance and quality monitoring of water resources intended for DW production and on treatment systems;
- the strategy for surveillance and quality monitoring of recreational water;
- the management measures to be implemented according to the results of surveillance and/or quality monitoring.

3.1. Identification of potentially toxigenic cyanobacteria

Cyanobacteria can be identified under a light microscope on the basis of numerous reference books, manuals or taxonomic identification keys. However, there is no French reference enabling these micro-organisms to be identified in a uniform way. The main disadvantage of consulting different literature sources is that they can be a major source of inconsistencies, especially if identification is taken to the species level. For this reason, the experts drew up a list of the taxa producing the various cyanotoxins found to date in freshwater in metropolitan France and the French overseas territories and which have proven toxicity for aquatic or terrestrial vertebrates, stopping at the genus level (Table I). Determination of the genus is essential to identify potential toxicity, even though this toxicity can vary significantly between genotypes, and therefore between strains of cyanobacteria.

This list was compiled on the basis of a review and analysis of the recent scientific literature. It should be noted that the classification of cyanobacteria and knowledge of their toxic potential is regularly revised. The current list of toxin-producing cyanobacteria may therefore be modified due to advances in scientific knowledge, particularly in genomics, physiology and classification.

To supplement this list, the experts recommend continuing to identify planktonic and benthic cyanobacteria, and the toxins they produce, in freshwater used as a resource for DW production and in recreational water, particularly in the overseas territories, whenever a cyanobacterial bloom is confirmed.

Table I: Cyanotoxin-producing taxa in freshwater and seawater with proven toxicity to aquatic or terrestrial vertebrates

(Note: the genera identified in the table do not systematically produce toxins)

Toxins	Environment	Morphotype	Main genera of proven toxin-producing cyanobacteria	Other genera of proven toxin-producing cyanobacteria
Microcystins	Freshwater and terrestrial environments (cyanobacteria in symbiosis with fungi to form lichens), brackish estuarine water	Unicellular colonial	*Microcystis*	*Aphanocapsa*, *Merismopedia*, *Radiocystis*, *Woronichinia*
		Filamentous	*Planktothrix (Oscillatoria)*	*Annamia*, *Geitlerinema*, *Leptolyngbya*, *Limnothrix*, *Kamptonema/Phormidium/Microcoleus*, *Pseudanabaena*, *Spirulina*, *Trichodesmium*, *Plectonema*
		Filamentous with heterocyst	*Anabaena*	*Anabaenopsis*, *Calothrix*, *Nostoc*, *Trichormus*
		Filamentous with heterocyte and branching	*Hapalosiphon*	*Fischerella*
Anatoxin-a	Freshwater	Filamentous with heterocyte	*Anabaena*	*Aphanizomenon, Cuspidothrix, Cylindrospermum, Dolichospermum,* *Raphidiopsis/Cylindrospermopsis*
		Filamentous	*Kamptonema/Phormidium/Microcoleus* *Oscillatoria* *(benthic organisms)*	*Pseudanabaena*, *Tychonema*
Anatoxin-a(S)	Freshwater	Filamentous with heterocyte	*Dolichospermum* (*Anabaena*)	
Cylindrospermopsins	Freshwater	Filamentous with heterocyte	*Raphidiopsis/Cylindrospermopsis*	*Aphanizomenon*, *Anabaena*, *Raphidiopsis*, *Dolichospermum*, *Chrysosporum*
		Filamentous with heterocyte and branching	*Umezakia*	
		Filamentous	*Kamptonema*/*Phormidium*/Microcoleus *Oscillatoria*	*Lyngbya*

Toxins	Environment	Morphotype	Main genera of proven toxin-producing cyanobacteria	Other genera of proven toxin-producing cyanobacteria
			(typically benthic organisms)	
Saxitoxins	Freshwater (and seawater, produced by other organisms)	Filamentous with heterocyte	*Aphanizomenon*	*Anaebaena, Dolichospermum, Raphidiopsis/Cylindrospermopsis, Cuspidothrix, Raphidiopsis, Scytonema*
	Seawater	Filamentous	*Lyngbya (typically benthic organisms)*	*Hydrocoleum, Trichodesmium*
Beta-methylamino-L-alanine (BMAA)	Seawater	Filamentous	*Leptolyngbya*	
Nodularins	Sea/brackish water (and freshwater according to Foss *et al.,* 2016)	Filamentous with heterocyte	*Nodularia*	*Nostoc*
Lyngbyatoxins	Seawater	Filamentous	*Lyngbya (typically benthic organisms)*	*Moorea (Moorena)*
Aplysiatoxins	Seawater	Filamentous	*Lyngbya (typically benthic organisms)*	*Moorea (Moorena), Leibleinia*
Palytoxins	Seawater	Filamentous	*Trichodesmium*	

Note:
Genus names in brackets correspond to synonyms and redistributed taxa (e.g. some of the genetically related *Anabaena* and *Aphanizomenon* form the new taxon *Dolichospermum*) while genus names separated by '/' correspond to morphospecies complexes whose identification and naming may currently be controversial. The classification of these organisms is also evolving, particularly in light of knowledge recently acquired about the genomes and the evolutionary and adaptive history of these organisms.

3.2. Routes of exposure to cyanotoxins and cases of poisoning reported in France

Water from a resource experiencing a cyanobacterial bloom can potentially contain cyanotoxins, released mainly through cell death and partially through excretion. The amount of toxin(s) produced by a given population is highly variable during a bloom, depending on the growth dynamics of the population and the dynamics of toxin production by cells with the genetic material to produce it.

Swimming, water sports, drinking water and eating contaminated fish are all sources of human exposure to cyanotoxins.

The effects of cyanotoxins on human health vary according to the compounds involved. Fever and gastrointestinal disorders (nausea, vomiting) are the most frequently reported effects. However, eye or skin irritation and rashes are also described, along with myalgia, and liver or kidney damage.

The time to onset of symptoms is also highly variable because it depends on the type of toxin involved, the dose and the route of exposure. It can range from a few minutes to a few hours for skin symptoms and neurological disorders, and can be up to several hours for gastrointestinal disorders.

In France, 95 cases of human poisoning by cyanobacteria were recorded by poison control centres (CAPs) between 1 January 2006 and 31 December 2018. The majority of these cases occurred in the period 2016-2018 (13 cases in 2016, 12 cases in 2017 and 16 cases in 2018), mostly during the summer (June, July and August). This number is probably vastly underestimated due to a lack of awareness of this phenomenon by the general public and to non-specific symptoms, which in addition can disappear quickly and are not necessarily reported by people to doctors and health authorities. In addition, even when the diagnosis is suggested, a lack of investigation means that it cannot always be confirmed. Of the cases identified by the CAPs, 58 were symptomatic of intoxication[4] with cyanotoxins, although the level of causality between symptoms and exposure is often difficult to establish with certainty due to the absence of associated metrological data (Greillet *et al*., 2020).

The majority of intoxication cases reported over the last three years have been observed north of the Loire in summer, and in the context of bathing or water sports. Only three people were exposed as a result of consuming food. The cases described primarily concern children and young adults, which corresponds to the group most exposed during aquatic activities, with mainly digestive, dermal and neurological/neuromuscular symptoms. No serious cases requiring hospitalisation have been reported.

In recent years, it has been mainly cases of animal intoxication (especially dog deaths) that have attracted the attention of the authorities and media.

In order to improve the monitoring of intoxication cases, the experts therefore recommend:

1) **developing the means and tools for collecting cases of cyanobacterial intoxication and/or cases of intoxication by suspected or confirmed toxins. Ties between the Regional Health Agencies (ARSs) and the CAPs should be strengthened. In order to carry out this epidemiological vigilance, the professionals concerned (doctors, veterinarians, pharmacists) should be (i) made aware of the possibility of this aetiology when faced with intoxications and (ii) encouraged to report suspected or confirmed cases to the health authorities (ARSs and CAPs);**
2) **developing an investigation reference standard for validating cases of animal and human intoxication by cyanotoxins;**
3) **conducting an epidemiological study of the risks associated with exposure to cyanobacteria and identifying the cyanotoxin(s) involved in intoxication.**

[4] state of an organism after ingestion of an excessive quantity of toxins.

3.3. Toxic effects of cyanotoxins, toxicity reference values

Studies on the toxicity of cyanotoxins in freshwater mainly concern exposure through ingestion of water. Very few studies have investigated exposure by inhalation or dermal contact. It was therefore not possible to establish a toxicity reference value (TRV) that could be used to characterise the hazards and risks specific to these two exposure routes for all the cyanotoxins considered.

Considering that in France, DW production systems are generally able to eliminate both cyanobacterial cells and toxins present in water resources, chronic exposure to cyanotoxins seems unlikely today. On the other hand, repeated exposure over a few months cannot be ruled out, particularly in view of the seasonal nature of bathing activities.

Microcystin-LR
The new toxicological data available since the Agency's previous work in 2006 were used to update the TRV for MC-LR. Several recent studies have shown effects on the male reproductive system at lower oral doses than in the study used so far by the WHO to derive the TRV and propose management thresholds. A subchronic oral TRV based on altered sperm quality in mice, including decreased sperm motility, decreased sperm count and increased sperm abnormalities, was therefore developed (ANSES, 2019). This value is associated with a moderate confidence rating. It should be noted that it was not possible to use the available toxicological studies to establish an acute TRV for MC-LR.

Cylindrospermopsin
In rodent studies, the effects most sensitive to CYN (occurring at the lowest tested doses) from subchronic oral exposure are observed in the liver and kidneys. Using recent literature data, a new subchronic oral TRV based on increased liver and kidney weight was established (ANSES, 2019). This value is associated with a moderate confidence rating.

Saxitoxin
The main toxic effect of STX and its variants is neurotoxicity. Based on experimental studies in mice, the most sensitive effects (occurring at the lowest tested doses) during acute oral exposure to STX are manifested by abdominal breathing, lethargy, and decreased exploratory behaviour of the animals (Munday *et al.*, 2013). A new acute oral TRV was established for STX, selecting as the critical effect the dysfunction of skeletal muscles, which reflects neurological disorders caused by the blocking of voltage-gated sodium channels (ANSES, 2020). A low confidence level was assigned to this TRV.

Anatoxin-a
It was not possible to establish an acute oral TRV for ATX-a because the toxicological data currently available are too limited to characterise the hazard for humans. Nevertheless, ATX-a generally causes rapid paralysis of the muscles and respiratory system of intoxicated organisms.

The TRVs selected in this expert appraisal to characterise the risk from cyanotoxins in freshwater and fish are summarised in Table II.

1 **Table II: Selected toxicity reference values for three cyanotoxins found in freshwater and freshwater fish**

Cyanotoxin	Route and duration of exposure	Critical effect (key studies)	Critical concentration	Uncertainty factor	TRV
Microcystin-LR CAS no. 101043-37-2	Oral subchronic	Altered sperm quality Chen *et al.* (2011)	NOAEL[5] = 1 µg.L^{-1} = 0.15 µg.kg bw^{-1}.d^{-1}) Allometric adjustment[6] $NOAEL_{HED}$ = 0.02 µg.kg bw^{-1}.d^{-1}	25 UF_A: 2.5 UF_D: 10	**1 ng.kg bw^{-1}.d^{-1}** Confidence level Moderate
Cylindrospermopsin CAS no. 143545-90-8	Oral subchronic	Increased liver and kidney weights, correlated with histological and biochemical damage Chernoff *et al.* (2018)	LOAEL = 75 µg.kg bw^{-1}.d^{-1} Allometric adjustment $LOAEL_{HED}$ = 10.31 µg.kg bw^{-1}.d^{-1}	75 UF_A = 2.5 UF_H = 10 UF_L = 3	**0.14 µg.kg bw^{-1}.d^{-1}** Confidence level Moderate
Saxitoxin CAS no. 35523-89-8	Oral acute	Skeletal muscle dysfunction Munday *et al.* (2013)	NOAEL = 164 µg.kg bw^{-1} Allometric adjustment $NOAEL_{HED}$ = 22 µg.kg bw^{-1}	250 UF_A: 2.5 UF_D: 10 UF_H: 10 UF_L: 1 UF_S: 1	**0.1 µg.kg bw^{-1}.d^{-1}** Confidence level Low

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[5] NOAEL: No observed adverse effect level; LOAEL: Lowest observed adverse effect level

[6] Allometric adjustment: calculation of the equivalent dose or concentration for humans in the case of an animal study

Since the literature review showed that documentation on the toxic effects of cyanotoxins is still very sparse, the experts recommend developing research efforts and acquiring knowledge, in particular on the following topics:

1) **the acute toxicity of ATX-a, in order to develop an acute oral TRV;**
2) **the acute toxicity of MC-LR, in order to develop an acute oral TRV;**
3) **the toxicity of STX, from both acute and (sub)chronic oral exposure;**
4) **the toxicity of the different known cyanotoxin variants;**
5) **the acute and (sub)chronic toxicity of mixtures of cyanotoxins;**
6) **the potential toxicity of the many other metabolites produced by cyanobacteria;**
7) **the nature of the compounds and the mechanisms causing dermal toxicity and skin irritation.**

3.4. Hazard control

As stated above, cyanotoxins remain largely intracellular until the lysis of cyanobacterial cells. Accordingly, this opinion considers cyanobacteria potentially producing these toxins to be agents of cyanotoxin contamination of DW and recreational water, and agents posing a risk of exposure to these toxins. Controlling the risks associated with cyanotoxins therefore inevitably involves controlling the development of the cyanobacteria that produce them. To the best of our knowledge, so far, deliberations and actions relating to this control have exclusively concerned planktonic cyanobacterial blooms. As these are linked to the eutrophication status of water bodies, the experts insist that long-term control of nutrient inputs – particularly phosphorus and nitrogen – to water bodies is the only lasting solution to limit planktonic cyanobacterial blooms. Short-term solutions based on chemical, biological and physical processes suggested to water resource managers to reduce and/or eliminate blooms on an ad hoc basis often produce unsatisfactory results, with poorly documented environmental effects.

The experts therefore believe that physical processes such as those designed to destratify the water column or hypolimnetic oxygenation[7] are not suitable for all lakes and should only be implemented as a transitional measure and to supplement actions designed to directly reduce nutrient inputs.

Furthermore, the experts are not in favour of chemical treatments of water bodies in natural environments, whether preventive or curative, for the following reasons:

- risk of release of cyanotoxins into the water (in resources, raw water storage ponds or in treatment systems);
- lack of knowledge or incomplete assessment of the impacts of such products on the fauna and flora present.

If these treatments are used nonetheless, the experts reiterate that all algicides must have received marketing authorisation according to the European Regulation concerning the making available on the market and use of biocidal products (BPR, Regulation (EU) No 528/2012).

The experts also point out that according to Article R.1321-43 of the French Public Health Code, it is not possible to use treatment products and processes directly in water bodies used for DW production.

The WG also mentions that the quality of raw water used for DW production must be continuously monitored to prevent hazards associated with the presence of toxins in DW. Vulnerability to cyanobacteria/cyanotoxins should be taken into account when establishing water safety management plans.

If cyanobacterial blooms are observed in the resource, it is necessary to adapt the treatment in the system as far as possible and reinforce surveillance of cyanobacteria and their toxins. The experts recommend, wherever possible, the use of a variable-height water intake, to enable water to be pumped from layers that are less contaminated with cyanobacteria.

If the resource is regularly subject to cyanobacterial blooms, a system based on a "multi-barrier" treatment including a combination of steps based on different principles should help control the risk associated with the presence of toxins in DW.

The experts recommend initially using physico-chemical clarification, which is highly effective at eliminating cyanobacterial cells. Dissolved air flotation is the separation technique recommended by the

[7] Technique designed to avoid the establishment of an anoxic zone at depth in order to prevent the release of phosphorus from sediments

experts during physico-chemical clarification for treatment systems pumping a resource that is regularly subject to cyanobacterial blooms. Once the cells have been removed, the experts recommend one or more refining treatments. Adsorption on powdered activated carbon should be preferred because the dose of activated carbon to be added can be easily adapted to the concentration of dissolved toxins[8] in the water to be treated. Nanofiltration or reverse osmosis using membranes with a cut-off point below the molar mass of cyanotoxins are also recommended by the experts as a refining treatment. Chemical oxidation treatments (ozone, chlorine, chlorine dioxide) can degrade toxins but they must be applied to water with a low organic matter content (TOC < 2 $mg.L^{-1}$) because high doses of these oxidants can lead to the formation of by-products (oxidation by-products classically screened for in DW that can be hazardous to human health).

The experts insist on the need for a good understanding of the different treatment steps by managers, and recommend that if cyanobacterial blooms are observed in raw water, the following operational measures be taken in treatment systems:

- stopping the pre-oxidation steps, to avoid releasing intracellular toxins into the water to be treated;
- adapting the treatment steps and reagent doses to be used:
    - optimising the dose of coagulant/flocculant to ensure cell removal by coagulation/flocculation and decantation or flotation;
    - adapting the dose of powdered activated carbon according to the concentration of dissolved toxins;
    - adapting the doses of oxidant (ozone, chlorine, chlorine dioxide) to satisfy demand while maintaining the required disinfecting properties.

The experts also recommend reinforced monitoring of the operating parameters of the treatment systems and in particular:

1) **regular extraction – and therefore reduction in residence times – of sludge from settling tanks to prevent possible lysis of cyanobacteria accumulated in the sludge, which could lead to the release of intracellular toxins;**
2) **continuous monitoring of filter head loss and turbidity of filtered water, to avoid puncturing of the filters, which could lead to the release of large numbers of cells into the filtered water;**
3) **optimisation of filtration cycles to avoid the release of intracellular toxins into the filtered water (from cyanobacterial cells accumulated in the filter material);**
4) **ceasing the recycling of filter backwash water if this water is not treated by an effective adsorption treatment before being re-injected into the head of the system. If this water undergoes specific treatment, it is necessary to adapt the treatment rate and verify its effectiveness;**
5) **adapting the regeneration frequency of the granular activated carbon in the event of recurrent blooms.**

3.5. Review of the presence of cyanobacteria and cyanotoxins in France

The results of analyses conducted over the period 2010-2017 by the official laboratories approved for quality monitoring of DW and bathing water and fed into respectively the national SISE-Eaux database (environmental health information system) and the SISE-Baignade database, were extracted in order to obtain a view of contamination of French water bodies by cyanobacteria. More than 80,000 results were extracted from the SISE-Eaux database and nearly 160,000 from the SISE-Baignade database. Various difficulties were encountered while analysing these two databases, due to (i) heterogeneity in the information associated with each sample and analysis, (ii) a heterogeneous distribution of data across the country and a virtual absence of data for the overseas territories, and (iii) a very heterogeneous temporal distribution of analyses from one *département* to another. This meant that regardless of which

8 Toxins found in raw water or released after cell lysis from the previous treatment steps

database is considered, SISE-Eaux or SISE-Baignade, the results are difficult to compare and not fully exploitable.

As a result, the data extracted on cyanotoxins are currently insufficient for estimating exposure of the French population to the various cyanotoxins via DW or recreational water. In fact, when cyanotoxin concentration results are available, the information is biased by the fact that screening for toxins is only carried out when the cyanobacteria threshold is higher than the regulatory threshold in DW or bathing water. In addition, the analytical methods used for detecting toxins differ between laboratories and therefore do not provide the same level of information.

Although the statistical processing of quality monitoring data could not be used to precisely map the contamination of water bodies by cyanobacteria in France, a certain amount of information has nevertheless emerged. In particular, the genera most frequently observed in bathing water and in catchment water (*Anabaena, Aphanizomenon, Aphanocapsa, Aphanothece, Microcystis*, *Planktothrix, Pseudanabaena, Woronichinia*) are, with the exception of just one (*Aphanothece*), all potentially toxigenic. They are likely to produce toxins belonging to the main families of cyanotoxins: MC, ATX, CYN and STX.

The collection of data on the contamination of rivers and water bodies therefore needs to be continued and improved, as it is an essential step in estimating the exposure of populations in metropolitan France and the overseas territories, regarding both the consumption of DW and the practice of freshwater recreational activities.

To improve the relevance of the data collected and their use, the experts recommend:

- regarding the organisation of data collection and storage:
    1) **harmonising the collection of surveillance and quality monitoring data by standardising the surveillance parameters and data storage rules;**
    2) **supplementing the data on abundance entered for each cyanobacteria genus with biovolume data using the standard biovolumes assigned to each of the genera (see Annex 1 to this opinion);**
    3) **setting up monitoring of all cyanotoxins in freshwater;**
    4) **promoting quality monitoring in the French overseas regions in order to estimate cyanobacteria and cyanotoxin contamination in these regions;**

- regarding the acquisition of knowledge:
    1) **continuing research on the contamination of brackish water by cyanobacteria and cyanotoxins;**
    2) **assessing the risk of cyanobacteria and cyanotoxins being transferred along the freshwater/estuary/seawater continuum.**

3.6. Review of regional health agencies' practices in quality monitoring and health management of cyanobacterial blooms and cyanotoxins

A survey aimed at taking stock of the practices of regional health agencies (ARSs) in terms of surveillance and management of cyanobacterial blooms and cyanotoxins was drawn up by the "Cyanobacteria" WG and sent to all ARSs by the DGS. It covered DW, bathing and recreational water, and addressed the following points: existence of surveillance of cyanobacteria and/or their toxins, organisation of quality monitoring (parameters, period and frequency, strategy, analytical techniques, cost), methods for storing results, health management strategy, existence of health signals and lastly management difficulties. In total, responses to the questionnaire were obtained for 68 *départements*, 67 of which were in metropolitan France and one in the overseas territories.

These responses highlighted:

- major disparities in the implementation of quality monitoring of cyanobacteria and/or their toxins depending on the *département*, making it impossible to obtain an overview of the situation at national level. This monitoring is non-existent in some *départements* and in those where it is practised, the methods and costs of implementation vary greatly;

- timeframes for sending cyanobacteria counts and cyanotoxin analysis results from analytical laboratories to the ARSs that are often incompatible with health management requirements;
- a high degree of heterogeneity in the storage of results, which complicates exploitation of the SISE-Eaux and SISE-Baignade databases;
- wide variability in health management practices and difficulties in implementing this management.

To overcome these disparities between *départements*, the experts recommend harmonising practices at the national level, based on the definition of a surveillance strategy that takes into account the most recent scientific knowledge on cyanobacteria and their toxins. With this harmonisation in mind, the detailed decision trees presented below (Figures 1, 2 and 3) were developed by the "Cyanobacteria" WG for surveillance of both DW and bathing water.

3.7. Estimate of the health risks

There are insufficient data on cyanotoxin concentrations collected in the SISE-Eaux and SISE-Baignade databases to be able to establish robust average concentration values that could then be used to characterise the health risk associated with ingestion of these compounds via drinking water or bathing water. In this context, maximum tolerable concentrations (MTCs) of cyanotoxins in DW and recreational water were estimated to ensure that exposure would be below the toxicity reference value in the case of single ingestion (acute exposure for ATX and STX) or repeated ingestion over time (subchronic exposure for MC and CYN). These MTCs were derived from the TRVs and exposure scenarios selected by the "Cyanobacteria" WG.

Besides ingestion, other routes of exposure to cyanotoxins are possible (inhalation, mucocutaneous contact) but were not considered in the determination of MTCs. The guideline values were established while assuming a 100% share attributable to DW and recreational water.

Tables III and IV summarise all the parameter values used to calculate MTCs in DW and recreational water.

Table III: Acute and subchronic exposure scenario for DW

Populations	Body weight (in kg)	Ratios of total daily water consumption to body weight (at P95)[1] (L/kg bw/day)	Duration of acute exposure (in days)	Duration of subchronic exposure (in days)
Child up to 6 years of age	15	0.131	1	30
7-10 years	29	0.059	1	30
11-14 years	46	0.053	1	30
15-17 years	62	0.030	1	30
Adult over 18 years of age	70	0.031	1	30

[1] Data from the INCA3 study (ANSES, 2019c); P95: 95th percentile of the distribution

Table IV: Acute and subchronic exposure scenario for recreational water

Populations	Body weight (in kg)	Volume of water ingested per bathe (in mL)	Duration of acute exposure (in days)	Duration of subchronic exposure (in days)
Child up to 6 years of age	15	50[1]	1	15 or 30
7-10 years	29	30[2]	1	15 or 30
11-14 years	46	30[2]	1	15 or 30
15-17 years	62	18[2]	1	15 or 30
Adult over 18 years of age	70	7[2]	1	15 or 30

[1] Owen and Sunger, 2018 – [2] DeFlorio-Barker *et al.*, 2018

These data show that the ratio of body weight to water intake is highest for children under six years of age. The proposed guideline values not to be exceeded for DW and recreational water in Table V are therefore the MTCs calculated for children under six years of age.

As TRVs are not available for all the variants of each toxin, the proposed guideline values are the sum concentration of all variants of each toxin not to be exceeded.

The MTC value calculated for MCs in DW is 8 $ng.L^{-1}$ for children under six years of age. Following the example of the WHO for certain micropollutants (e.g. bromates), the experts propose using the limit of quantification of the official laboratories in France as the maximum admissible concentration. This limit of quantification is currently 0.2 $\mu g.L^{-1}$ for MCs (communication from ANSES's Nancy Laboratory for Hydrology).

Similarly, in the absence of a TRV for ATX-a, the experts recommend verifying, during quality monitoring, that ATXs are not detectable.

Table V: Proposed maximum tolerable concentrations of cyanotoxins for DW and recreational water

	Microcystins* (in $\mu g.L^{-1}$)	Cylindrospermopsins* (in $\mu g.L^{-1}$)	Saxitoxins* (in $\mu g.L^{-1}$)	Anatoxins*
DW	0.2	1	0.8	< LD
Recreational water	0.3	42	30	< LD

* Sum of the variants screened for and quantified

3.8. Detection and quantification of cyanobacteria and cyanotoxins

As cyanotoxins are not excreted to a significant degree by the cyanobacteria that produce them and therefore remain in the cells until their senescence and lysis, the health risk associated with cyanotoxins is closely linked to the risk of exposure to toxigenic cyanobacteria. As a result, detection and quantification of cyanobacteria on the one hand and of cyanotoxins on the other are two necessary approaches to health risk assessment.

3.8.1. Detection of cyanobacterial blooms

The main difficulty inherent in detecting blooms and quantifying cyanobacteria that may produce and contain cyanotoxins is the heterogeneity of their distribution in water bodies and rivers. Indeed, with both planktonic and benthic cyanobacteria, there can be large variations in their spatial and temporal distribution in water bodies, linked, among other things, to the life cycle of the species, transport by

currents or winds, circadian migration or predation. Optimising sampling and quantification protocols is therefore a fundamental challenge for the detection and quantification of cyanobacteria.

The literature review indicated that detection of cyanobacterial blooms, both planktonic and benthic, should begin with visual observation. The use of keys to recognising different types of bloom is then recommended (Annex 2).

For planktonic cyanobacteria, visual surveillance of water bodies (DW resources or recreational waters) should be coupled with measurements, in the environment or in the abstracted water, of changes in photosynthetic pigment concentrations. This involves regularly measuring, *in situ* or in the laboratory, the concentration of total chlorophyll-a (i.e. attributed to the entire phytoplanktonic biomass) and/or the concentration of chlorophyll-a equivalent attributed to cyanobacteria and/or the concentration of accessory pigments specific to cyanobacteria (phycocyanin, phycoerythrin). For resources intended for DW production, monitoring by the entity responsible for water production and distribution (PRPDE) of indicators such as pH, dissolved oxygen or turbidity (parameters that can be measured continuously *in situ*) can provide additional information useful for the detection of blooms, thereby improving management of the treatment system.

Detection of cyanobacterial blooms should also rely on samples of water (for planktonic cyanobacteria) and biofilms (for benthic cyanobacteria) in order to verify the presence of cyanobacteria using light microscopy, identify the genera present and assess the abundance of potentially toxic genera. If necessary, these samples could also be used to determine cyanotoxin concentrations in cyanobacteria (intracellular cyanotoxins) and in water (free cyanotoxins).

For the detection of benthic cyanobacterial blooms, visual observation will help define the areas and periods of biofilm development, as well as the periods favourable to biofilm detachment and then the areas in which they accumulate after being transported by the river. Carrying out these observations requires training in biofilm recognition and the use of illustrated fact sheets. The dominance of cyanobacteria in the biofilms can then be confirmed by light microscopy. The episodes of benthic cyanobacteria growth on the Loire and Cher rivers during the summers of 2017 and 2019 showed that estimating the rate of substrate cover at the bottom of rivers (as recommended in New Zealand where river beds are mainly rocky, consisting of pebbles and boulders) is not always suitable for assessing the extent of a benthic cyanobacterial bloom in these rivers. Indeed, in the Loire and Cher rivers, it was observed that biofilms often developed on macrophytes floating in the current, or on areas of sandy and loose soil. In this situation, estimating coverage rates is more difficult and these rates can also vary over a very short time with even slight increases in currents, which can lead to the detachment and suspension of biofilms. The coverage rate is then reduced, but the risk is still present because the biofilms are circulating in the water body and can accumulate on the banks.

3.8.2. Sampling of cyanobacteria

When taking samples, a field sheet should be completed. In addition to the geographical coordinates of the surveillance point and the location from which the samples were taken, this sheet should include all information on the site and its environment at the time of sampling (colour of the water or bottom, wind direction and intensity, presence or absence of coloured deposits on the bank, agglomerates/flocs in suspension or on the surface, or any other relevant observation such as the presence of dead fish, unpleasant odours, etc.). An example of a field information sheet is given in Annex 3.

In order to harmonise practices, a provisional methodological guide on cyanobacteria sampling in fresh water used for bathing and water sports was distributed widely to health authorities and laboratories responsible for sampling and analysis for quality monitoring in 2016 (ANSES, 2016b). However, not all the provisions in this methodological guide are suited to the resources used to produce drinking water or to sampling of water leaving the drinking water purification plant. Nor does this guide take account of the specificities of benthic cyanobacteria. This document should therefore be completed with the additional information defined below.

Because of their different growth strategies (presence in the water column for planktonic cyanobacteria or development on a substrate for benthic cyanobacteria), sampling of planktonic and benthic cyanobacteria cannot be carried out in the same way. For this reason, these two categories will be addressed separately below.

3.8.2.1 Sampling and storage of planktonic cyanobacteria and cyanotoxins

3.8.2.1.1 Equipment and bottles

Sampling equipment

Various sampling devices and methods are currently used in France for the different water bodies. In order to optimise and harmonise sampling practices, the "Cyanobacteria" WG recommends the use of a 1 m long sampling tube with a minimum volume of 250 mL that can be manipulated with one hand to take samples.

Bottles

For samples intended for chlorophyll-a analysis, opaque polyethylene or brown glass 1 L bottles should be used. They should be filled completely so that all air is expelled. For cyanobacteria counts, two polypropylene (PP) or glass bottles of at least 200 mL (ideally 500 mL) should be used. Amber glass bottles of at least 200 mL (ideally 500 mL) should be used for sampling with a view to subsequent analysis of toxins.

3.8.2.1.2 Sampling in bathing and water sports areas

In bathing and water sports areas, at least one composite sample is recommended. This corresponds to a mixture of samples taken from at least three sampling points, evenly distributed over the monitored area.

At each sampling point, the sample should be taken from the first metre of the water column with the sampling tube. Depending on the volume of the sampling tube, it may be necessary to take several successive samples in order to obtain the necessary volume for the subsequent analyses.

All the samples should be mixed in a bucket (previously rinsed with water from the environment) from which the sample(s) for analysis should then be taken. The sample(s) should be taken immediately after the contents of the bucket have been homogenised. These samples will be used for identification and counting of cyanobacteria, chlorophyll-a determination and possibly toxin testing if warranted by the results of the count.

In the event of a bloom, if an accumulation area is observed in one part of the water body, a sample could be taken from a fourth point in this specific area. In this case, the sample should not be mixed with the others so that it can be processed separately.

3.8.2.1.3 Sampling of surface water resources used for DW production and within treatment systems

For resources used for DW production, the surveillance strategy deployed by the operator (surveillance) should be optimised according to the objective sought: representativeness of the quality of the surface water that will be used for the supply, or warning of the beginning of a bloom that could affect the resource. It will therefore be important, during bloom episodes, to sample water at different points in the resource and at different depths, in order to determine the horizontal and vertical distribution of cyanobacteria. Each sample should be analysed individually and therefore no composite samples should be taken in this case.

For the DW quality monitoring carried out by the ARSs, it is generally considered sufficient to take a sample corresponding to the resource and a sample from the water distribution point, for the purpose of toxin analysis. These samples should be taken directly with a bottle or sampling tube and, as mentioned above, all the samples should be analysed separately (counts of cyanobacteria and determination of chlorophyll-a concentrations and, if necessary, toxins).

Within the treatment systems, in the event of a bloom on the resource, samples should be taken at different treatment steps (decanted water, filtered water). These samples should generally be taken at different steps of the process using specially designed taps. Cyanobacteria and cyanotoxins can be screened for in every sample taken. For samples taken after a chemical disinfection step (ozone, chlorine), the bottle used should contain sodium thiosulphate to neutralise any residual oxidant. This addition should be mentioned in the analysis protocol.

3.8.2.1.5 Storage and transport

The storage and transport guidelines described in the XP T90 719 Standard on sampling of phytoplankton from inland waters are largely applicable to cyanobacteria samples.

Accordingly, samples intended for the identification and counting of planktonic cyanobacteria must be fixed with alkaline Lugol's solution at the time of collection, at a concentration of 0.5% by volume (16 drops for a 200 mL bottle filled to 80%, turning the water orange). In the laboratory, samples can be stored in a dark place at room temperature.

However, for samples intended for the quantification of toxins, no fixative should be added.

All samples should be transported in a refrigerated cabinet kept at a temperature of 5 +/- 3°C, according to the NF EN ISO 5667-3 Standard (on the preservation and handling of water samples), and in the dark until reaching the laboratory. If toxins are not analysed immediately, samples can be stored in a refrigerated cabinet for up to 36 hours after collection.

3.8.2.2 Sampling and storage of benthic cyanobacteria and cyanotoxins

3.8.2.2.2 Equipment and bottles

Sampling equipment

Sampling should be taken from the substrates (blocks, pebbles, sand, plants) while wearing gloves and using fine flat-tip tweezers.

Bottles

Biofilm samples for cyanobacterial identification may be placed in polypropylene (PP) tubes of 5 mL or larger. Those for toxin screening should be placed in 50 mL amber glass bottles or tubes.

3.8.2.2.3 Sampling in bathing and water sports areas

In areas where cyanobacterial biofilm growth or accumulation is visible, a minimum of three biofilm samples should be taken at different points in the growth area. If, in the same area, growth is observed on different substrates (e.g. pebbles and macrophytes), samples should be taken from these different substrates. Equivalent sized fragments of each biofilm collected should be pooled in a first tube for identification by microscopy and in a second tube for possible toxin screening. Biofilm samples for identification should be topped up with water so that they are fully immersed inside the tubes.

Samples should be taken from the substrate, either *in situ* directly in the water, or *ex situ*, where possible, after removing the biofilm substrate from the water to facilitate sampling.

3.8.2.2.4 Sampling of resources used for DW production and within treatment systems

In the current state of knowledge, as surface water catchment areas are not affected by benthic cyanobacteria, the "Cyanobacteria" WG does not recommend monitoring these cyanobacteria.

3.8.2.2.5 Storage and transport

Samples intended for the identification of benthic cyanobacteria and the determination of their dominance in biofilms must be fixed with alkaline Lugol's solution at the time of collection, adding a variable volume of Lugol's solution (depending on the density of the biofilms) to turn the water orange. In the laboratory, samples can be stored in a dark place at room temperature.

For samples intended for the quantification of toxins, no fixative should be added.

All samples should be transported in a refrigerated cabinet kept at a temperature of 5 +/- 3°C, and in the dark until reaching the laboratory. If toxins are not analysed immediately, samples can be stored in a refrigerated cabinet for up to 36 hours after collection.

3.8.3. Quantification of chlorophyll-a, identification and quantification of cyanobacteria

To avoid any delay in deploying the management measures to be taken by the PRPDEs and/or the entities responsible for the water bodies intended for recreational use, the analysis results should be made available very quickly. The "Cyanobacteria" WG recommends a maximum of 48 hours from the time of sampling for the transmission of results on chlorophyll-a concentration, taxonomic identification and cyanobacteria counts.

3.8.3.1 Quantification of chlorophyll-a in phytoplankton samples

Quantifying chlorophyll-a in the water body can provide an early warning of the establishment of a potentially toxic planktonic cyanobacterial bloom.

Chlorophyll-a analyses should be carried out in the laboratory by spectrophotometry in accordance with the NFT 90-117 Standard or by HPLC/UV in accordance with the NFT 90-116 Standard, in particular for quality monitoring. In addition, and especially as part of surveillance by operators, *in situ* probe measurements can be carried out (total chlorophyll-a, chlorophyll-a attributed to cyanobacteria or phycocyanin). Chlorophyll-a analyses are not necessary on water leaving the drinking water purification plant.

3.8.3.2. Identification and quantification of cyanobacteria by microscopy

3.8.3.2.1 Identification and quantification of planktonic cyanobacteria

The Utermöhl method (1958) using inverted microscopy after sedimentation of samples was standardised and normalised in Europe in 2006 (CEN 2006). This technique is classically used throughout the world and remains a reference. A study comparing its effectiveness with the method for counting and identifying cyanobacteria by upright microscopy with a Nageotte cell (Brient *et al.*, 2008), which received funding from the Agency, is under way (CRD-2018-CYAME). Pending its conclusions, the "Cyanobacteria" WG recommends the use of the Utermöhl method for counting planktonic cyanobacteria. In order to harmonise practices, the counting protocols proposed in Annex 4 should be followed.

To obtain a result in quantity of matter and not in cell count, the "Cyanobacteria" WG recommends converting the results of the counts into biovolume expressed in $mm^3.L^{-1}$. The aim is to estimate the associated cell volume for each genus in the sample, to avoid being constrained by the limitations of cell concentration, which does not take account of differences in cell size according to taxa and can lead to an overestimation of the relative importance of small genera. This method has been standardised at European level (Standard NF EN 16695) and integrated into counting tools.

To do this, the "Cyanobacteria" WG recommends the use of average cell biovolumes for each genus (Annex 1) in order to limit the problems of comparing biovolume estimation results. The biovolume of each genus found in the sample is calculated using the average cell volume specific to that genus, multiplying it by the number of cells counted per unit volume.

Calculating the biovolume of each genus in a sample requires a great deal of time and precision in the measurements, and is intended more for research purposes. For this reason, some free software tools such as Phytobs (Laplace-Treyture *et al*., 2017) incorporate an average biovolume per species/genus to facilitate their use.

Identifying and counting cyanobacteria are complicated steps requiring trained personnel. However, there are not enough limnologists and taxonomists to ensure reliable determinations. **Training on identifying and counting cyanobacteria should therefore be provided for laboratory operators.** These operators should be trained and allowed to participate in inter-laboratory tests (ILTs) in order to guarantee the quality of the results obtained. Obtaining test results, from sampling to counting, under accreditation by official laboratories, would also be a way of improving the reliability of the results.

3.8.3.2.2 Identification of benthic cyanobacteria

The cyanobacteria in the collected biofilms should be identified under an upright light microscope between the slide and coverslip.

However, the "Cyanobacteria" WG does not recommend quantifying benthic cyanobacteria in the biofilm samples collected, as this does not enable the biomass of these organisms to be assessed on the scale of the river area in question.

3.9. Detection and quantification of toxins

Regarding samples of benthic cyanobacteria, care should be taken to fully homogenise the sample resulting from the mixing of several biofilm fragments in a bottle before screening for toxins.

The "Cyanobacteria" WG recommends using ELISA as a quality monitoring method for the analysis of cyanotoxins in water, provided that a validated method is used. Of the commercially-available ELISAs, those with the highest level of cross-reactivity to the different variants should be preferred. Therefore, ADDA-specific ELISAs should be used for analysing MCs.

The choice to use the ELISA analytical method for cyanotoxin quality monitoring is based on the simplicity, speed, sensitivity and availability of kits for a broad spectrum of cyanotoxins, as well as the ability to process several samples simultaneously. Liquid chromatography with tandem mass spectrometry (LC-MS/MS), which is more specific and has the advantage of being able to identify and quantify the different variants for which standards are commercially available, is a complementary technique to ELISA that could be used for research purposes to produce additional data, pending the establishment of harmonised toxic equivalent factors at European and/or international level.

The "Cyanobacteria" WG also recommends beginning with screening for cyanotoxins in fish flesh using the ELISA method, provided that a validated method is used.

Regardless of the matrix considered, the "Cyanobacteria" WG stresses the importance of providing a minimum of information to assess the reliability of the analytical method in question (LOD, LOQ and extraction performance). The results of toxin analyses must be communicated within no more than 72 hours, regardless of the matrix considered.

The "Cyanobacteria" WG also recommends continuing research and development efforts in the following areas:

1) **Validating methods to promote harmonisation of surveillance and quality monitoring practices at national level;**
2) **Improving knowledge of the specific characteristics of the different methods used (e.g. according to AFNOR standards: limits of detection and quantification, extraction performance, matrix effects, internal repeatability and reproducibility). This step is necessary for the accreditation of each analytical method in order to guarantee the results obtained and obtain approval for the analytical laboratory;**
3) **Securing supplies in the long term and diversifying commercial sources of reference materials and standards, as well as ELISA kits for all cyanotoxin families;**
4) **Developing alternatives to the Lemieux oxidation used to analyse total forms (free and bound) of MCs, as this procedure has many methodological drawbacks.**

3.10. Cyanobacterial contamination of freshwater fish

3.10.1. Consumption frequencies not to be exceeded according to the concentration of microcystins and cylindrospermopsins in fish flesh

In order to provide useful guidance to managers for imposing or lifting bans on freshwater fish consumption in relation to blooms of toxin-producing cyanobacteria, the "Cyanobacteria" WG estimated, for cyanotoxins with subchronic effects (MC, CYN), consumption frequencies for fish flesh that should

not be exceeded according to the concentration of toxins measured, in order to limit exposure to levels below the toxicity reference value. For cyanotoxins with acute effects (STX), the WG estimated maximum concentrations not to be exceeded in fish flesh. These estimates are proposed for the general population, and have been subdivided into several categories according to the age of the individuals. This approach could not be followed for ATX-a due to the absence of a TRV, and the WG therefore advises against fish consumption whenever the concentration measured in water, or in biofilms, is above the analytical method's detection limit.

Consumption data were taken from the INCA2 and BEBE SFAE 2005 studies (detailed in the expert appraisal report). In the absence of any information on the greater sensitivity of children, the same TRV applies to the estimates for both adults and children.

3.10.1.1 Microcystin-LR

Table VI shows the freshwater fish consumption frequencies that should not be exceeded in order to maintain average exposure below the subchronic TRV of 1 ng/kg bw/d according to the level of MC-LR contamination in fish flesh, expressed as a concentration range (due to the measurement uncertainty, it is not possible to be more precise).

The table reads as follows: if the concentration of MC-LR is 50 µg.kg^{-1} in fish flesh, the consumption frequency not to be exceeded is "once every 2 months" for adults, "once every 3 months" for children aged 11 to 17 years, and "once or twice a year" for children aged 6 months to 10 years.

Table VI: Freshwater fish consumption frequencies not to be exceeded according to MC-LR contamination, for adults and children

		Adults	**Children aged 11 to 17 years**	**Children aged 4 to 10 years**	**Children aged 6 months to 3 years**
		MC-LR concentrations (µg.kg^{-1} fresh weight)			
Consumption frequencies	Once or twice a week	[0 – 5]	[0 – 3]	[0 – 2]	[0 – 1]
	Two to 3 times a month	]5 – 10]	]3 – 6]	]2 – 3]	]1 – 3]
	Once a month	]10 – 20]	]6 – 17]	]3 – 10]	]3 – 8]
	Once every 2 months	]20 – 60]	]17 – 30]	]10 – 20]	]8 – 17]
	Once every 3 months	]60 – 100]	]30 – 80]	]20 – 40]	]17 – 30]
	Once or twice a year	]100 – 500]	]80 – 400]	]40 – 200]	]30 – 150]

According to the literature data (ANSES 2016a and updated to August 2019), the highest reported average concentration of MC-LR in fish muscle in Europe was 119 ± 33 µg.kg^{-1} fresh weight by ELISA method, in carp in Greece (Papadimitriou *et al.*, 2012).

Note: The data from the literature show that the concentration of MC-LR in fish muscle is highly variable between individuals and over time (depending on the time of sampling in relation to the bloom). Taking several fish samples over time is therefore advised in order to identify the appropriate frequency to be recommended, which will also depend on the public health objective sought (the frequency may be according to the average concentration or the highest concentration).

3.10.1.2 Cylindrospermopsin

Table VII shows the freshwater fish consumption frequencies that should not be exceeded in order to maintain average exposure below the subchronic TRV of 140 ng/kg bw/d according to the level of CYN contamination in fish flesh, expressed as a concentration range.

The table reads as follows: if the concentration of CYN is 5 $mg.kg^{-1}$ in fish flesh, the consumption frequency not to be exceeded is "once a month" for adults and children aged 11 to 17 years, and "once every 3 months" for children aged 6 months to 10 years.

Table VII: Freshwater fish consumption frequencies not to be exceeded according to CYN contamination, for adults and children

		Adults	Children aged 11 to 17 years	Children aged 4 to 10 years	Children aged 6 months to 3 years
		CYN concentrations ($mg.kg^{-1}$ fresh weight)			
Consumption frequencies	Once or twice a week	[0 – 0.8]	[0 – 0.7]	[0 – 0.35]	[0 – 0.3]
	Two to 3 times a month	]0.8 – 3.5]	]0.7 – 3]	]0.35 - 1]	]0.3 – 1]
	Once a month	]3.5 – 6]	]3 – 5]	]1 - 2.5]	]1 – 2]
	Once every 2 months	]6 – 9]	]5 – 8]	]2.5 - 3.5]	]2 – 3]
	Once every 3 months	]9 – 16]	]8 – 14]	]3.5 - 7]	]3 – 5]
	Once or twice a year	]16 – 76]	]14 – 65]	]7 - 35]	]5 – 25]

The literature review (ANSES 2016 and updated to August 2019) identified only one study reporting CYN analysis in fish muscle in Europe. Concentrations measured by ELISA in two trout muscle samples in Italy were 0.1 and 0.8 $\mu g.kg^{-1}$ fresh weight (Messineo *et al.*, 2010).

Note: Taking several fish samples over time is advised in order to identify the appropriate frequency to be recommended, which will also depend on the public health objective sought (the frequency may be according to the average concentration or the highest concentration).

3.10.1.3 Maximum concentration of saxitoxin not to be exceeded in fish flesh (acute risk)

As the TRV for STX (0.1 µg/kg bw) is based on an acute effect that can occur after a single intake of food, the methodology followed for STX is different from that followed for MCs and CYN, whose TRVs are based on a subchronic effect. Instead of associating consumption frequency with cyanotoxin concentration, the maximum STX concentration not to be exceeded in fish flesh was estimated from serving sizes at the 95^{th} and 97.5^{th} percentiles of the population, for adults and children of different age groups (Table VIII).

The STX health threshold in fish can be defined by the risk manager according to the public health objective sought (P95 or P97.5 of the target population).

Table VIII: Estimated maximum STX concentration not to be exceeded for serving sizes at the 95th and 97.5th percentiles of the population, for adults and children of different age groups

		Age groups	Maximum STX concentration not to be exceeded (µg.kg^{-1} fresh weight)
P95 of the distribution of fish serving sizes by age group	84 g	Children aged 6 months to 1 year	11
	122 g	Children aged 1 to 3 years	10
	150 g	Children aged 4 to 10 years	17
	190 g	Children aged 11 to 17 years	28
	190 g	Adults	37
P97.5 of the distribution of fish serving sizes by age group	92 g	Children aged 6 months to 1 year	10
	150 g	Children aged 1 to 3 years	9
	180 g	Children aged 4 to 10 years	14
	200 g	Children aged 10 to 17 years	27
	224 g	Adults	31

The literature review (ANSES 2016a and updated to August 2019) did not identify any studies reporting STX analysis in fish muscle in Europe.

3.10.1.4 Anatoxin-a

Concerning the contamination of fish by ATX-a, the toxicity data are too limited to be able to characterise the hazard for humans. It was not possible to develop a health reference value. Nor was it possible, therefore, to issue recommendations on health thresholds. The mode of action reveals potent neurotoxicity, which has been implicated in episodes of animal mortality. ATX-a is a cholinergic agonist of nicotinic acetylcholine receptors. It induces neuromuscular blockade and skeletal muscle contraction. Acute toxicity in mice shows rapid effects with muscle paralysis and respiratory distress. In view of the acute toxicity of ATX-a, the WG advises against fish consumption whenever the measured concentration is above the analytical method's detection limit.

As part of a study carried out by the French Natural History Museum (MNHN) in 2017[9], fish were collected (2 bream, 2 roach, 2 mullet and whitebait) from two sites in the Loire and the concentration of ATX-a was measured by high-resolution mass spectrometry (UHPLC-HRMS) in the muscle, viscera and brain. These data showed that the concentration can reach 7642 µg.kg^{-1} fresh weight in roach muscle. Concentrations were even higher in roach brain, reaching 33,591 µg.kg^{-1}.

[9] Screening for cyanobacterial toxins (anatoxins and congeners) in water samples, biofilm matrices and fish matrices (report of 21 December 2017, 22 p).

3.10.2. Possibility of correlating health risk management thresholds for cyanobacteria in bathing water with the risk associated with freshwater fish consumption

Based on a systematic review of the literature, ANSES's scientific and technical support report entitled "Review of knowledge concerning the contamination of freshwater fish by cyanotoxins" (2016) had identified about 100 papers that could potentially provide useful information. This major summary work highlighted numerous deficiencies, particularly in knowledge of the contamination and elimination kinetics of cyanotoxins by fish, as well as in the potential link with the kinetics of cyanobacterial blooms.

This summary work had shown that:

- the vast majority of studies are on MCs;
- contamination of planktonivorous fish species seems to be higher than that of carnivores, although some studies tended to show the opposite;
- within the same species, smaller individuals appear more contaminated than larger ones;
- MCs accumulate preferentially in the liver and viscera, and to a lesser extent in muscle tissue;
- it was not possible to identify a simple relationship between MC contamination in muscle and the dose or duration of exposure to cyanobacteria in water;
- the elimination kinetics of cyanotoxins in muscle are not known and are a subject of controversy (i.e. MCs in muscle are eliminated in just a few days according to some studies, very slowly for others, while other studies have observed higher concentrations in muscle several days after cessation of exposure);
- protein-bound MCs may account for a very large proportion of total MCs[10], but information on their bioavailability and/or redistribution within organisms is still lacking (according to some authors, the mobilisation of these bound toxins from the liver to muscle could explain the increase in muscle concentrations sometimes observed after cessation of exposure).

In light of this work, it did not therefore seem possible to:

- establish a threshold of contamination of water with MCs or cyanobacterial cells below which contamination of fish muscle would not pose a health risk associated with consumption;
- identify a time frame for significant elimination of MCs from muscle after the cyanobacterial bloom episode.

The systematic review was updated in February 2019 and this identified 144 new scientific papers dealing with MC contamination of freshwater fish. A double reading of these studies led to eligible papers being selected on the basis of their relevance and of the reliability of the analytical methods, in order to extract the fish contamination data. In the end, 24 papers were deemed eligible of which only one had quantitative data that could be included in the database.

None of these new papers included information on the contamination kinetics of fish, either via water or food (trophic chain).

Regarding decontamination kinetics, one of the papers highlighted the complexity of the process of elimination of accumulated MCs in fish flesh, over a period of 90 days (Calado *et al*., 2018).

In addition, 16 of the 144 papers with data on MC concentrations in both water and freshwater fish were analysed. However, these studies only very rarely showed any concordance between the dates and/or location of the fish and water samples. It would therefore be unwise to look for a relationship between the concentrations in these two matrices (even assuming a simple relationship between them).

The conclusions of this systematic review of the literature therefore remain the same as those formulated by ANSES in 2016.

The lifting of any ban on fishing should therefore be based on an analysis of cyanotoxins in fish rather than on a post-bloom period of time.

[10] Greer *et al*. (2017) estimated that 85% of total MCs in tilapia muscle was in bound form.

The "Cyanobacteria" WG also recommends carrying out studies to acquire information on the contamination and elimination kinetics of cyanotoxins in fish and on the link with cyanobacterial blooms.

In order to acquire data on the relationship between the presence of cyanobacteria/cyanotoxins in water and the concentration of cyanotoxins in fish, the WG recommends conducting studies under controlled laboratory conditions and *in situ* in ecosystems. In the latter case, these studies should be based on joint sampling (location and time) of water and edible species of fish, taking their different diets into account. It would also be useful to study seasonal changes in cyanotoxin contamination in fish flesh.

Lastly, in view of the literature, which shows complex relationships between bound and free MCs, as well as organotropism that needs to be better determined, it seems necessary to take into consideration the free and bound forms of MCs that may be present in various organs (muscle, liver, viscera).

3.10.3. Monitoring methods to be implemented specifically to cover the food risk in addition to the monitoring of bathing water

Regarding the question on methods for monitoring fish, the methodology took the following into account:

- A primary objective to set up a fish sampling plan in order to estimate levels of cyanotoxin contamination (MC-LR, CYN, STX, ATX-a) in flesh.
- Data on MC-LR contamination of freshwater fish from a study in Lithuania (Bukaveckas *et al*., 2017). As the WG did not identify any French data on fish contamination, these data were used to illustrate the proposed approach.

In order to establish a sampling plan, the number of individuals to be sampled per species was defined using the equation below. Two sampling plans were drawn up: one taking into account and the other ignoring the data collection period (before, during and after the bloom).

$$n = \frac{|t|_{\alpha}^{2} \times \sigma^{2}}{(i \times \mu)^{2}}$$

- n: number of individuals needed
- σ^2: sample variance
- i: desired precision
- μ: sample average
- α: risk from the first species
- $|t|$: value of the Student's statistic at the 5% probability with the number of degrees of freedom depending on the number of species (under the assumption of a normal distribution)

The "Cyanobacteria" WG underlines the fact that a good knowledge of the water body (lake, aquaculture pond, river) is a prerequisite for implementing surveillance of fish in order to assess their contamination by cyanotoxins. This **initial study** should take into account the typology of the water body, the species of fish caught/consumed, the history (frequency, duration, intensity) of bloom episodes and measured cyanotoxins, the presence of benthic cyanobacterial biofilms, and the levels of cyanotoxins in fish flesh according to species or diet.

The data from this inventory can then be used to define a monitoring plan based on one or more sentinel species[11] of fish. As an illustration, the "Cyanobacteria" WG relied on data from the literature to show how such data could be used to estimate the number of fish specimens to be analysed according to thedegree of precision sought by managers (available in the expert appraisal report).

[11] A sentinel species (fish) becomes contaminated more quickly and at higher levels than other species, e.g. mussels are used by Ifremer as a sentinel species for monitoring the contamination of filter-feeding bivalve molluscs by marine biotoxins. A sentinel species must also be sufficiently abundant at the site, on a permanent basis, and must be easy to collect in adequate quantities for analysis.

To limit consumer exposure, the "Cyanobacteria" WG reiterates the general recommendations to remove the head and guts of fish before consumption (or before freezing) and to avoid consuming small fish whole (whitebait).

In order to be able to estimate the dietary exposure of consumers, the "Cyanobacteria" WG recommends acquiring data on:

- cyanotoxin (MC, CYN, STX, ATX-a) contamination of freshwater fish in France (species consumed, sampled throughout the year) as well as of other freshwater organisms consumed by humans (e.g. crayfish, frogs);
- consumption of freshwater fish in France (species, serving size, consumption frequency), as well as of other freshwater organisms such as crayfish and frogs;
- fishing practices (fishing areas, seasonality, whether or not the presence of bloom is taken into account, freezing of fish for consumption throughout the year, proportion of "catch-and-release" recreational fishing).

3.11. Surveillance strategy for resources used for drinking water production and health management

3.11.1. Planktonic cyanobacteria

Quality monitoring of resources used for DW production is carried out at two levels:

- surveillance by the operator (PRPDE). The parameters and monitoring frequencies are set by the operator;
- sanitary control, carried out by the ARSs. The monitored parameters and the analysis frequency are laid down by Order (Ministerial Order of 11 January 2007 on the sampling and analysis programme for sanitary control of water supplied by a public distribution network, in application of Articles R. 1321-10, R. 1321-15 and R. 1321-16 of the French Public Health Code, as amended).

At present, sanitary control recommends screening for MC in raw and treated water when visual and/or analytical observations show a risk of cyanobacterial blooms. Based on the hearings with resource managers and operators, it appears necessary for surface water bodies used for DW production to be monitored directly by the resource operator (surveillance), and for this to be supplemented by regular checks by the health authority (see decision tree, Figure 1).

The surveillance strategy for resources intended for DW production should be based on routine monitoring, reinforced in the event of suspicion (minimum threshold for vigilance) or confirmation (minimum threshold for alert) of cyanobacterial blooms. It should be accompanied by management measures if cyanobacterial blooms are observed in the resource used.

To this end, when drawing up water safety plans, the vulnerability of the resource to cyanobacteria and the ability of the treatment system to eliminate cyanobacteria and their intracellular or dissolved toxins in the water should be systematically characterised when the supply comes wholly or partly from surface water.

An inventory of all surface water resources used for DW production should be drawn up. Depending on the results of assessments carried out over a minimum period of three years, the frequency of sanitary control could then be adapted by the health authorities according to the vulnerability of the water intakes (for example, no monitoring if there is a total absence of cyanobacteria during these three years, or monthly monitoring only during the summer period).

- Operator surveillance and sanitary control

Surveillance by the operator

Cyanobacterial blooms are highly dynamic, evolving phenomena that appear more or less stochastically, in metropolitan France, most often between May and October, although they can occur throughout the year in the overseas *départements*. Because the factors and processes regulating cyanobacterial blooms are particularly complex, these phenomena are often difficult to predict. Bloom episodes may occasionally occur between the taking of two samples for quality monitoring purposes, and therefore go unnoticed by the health authorities. For this reason, it is important to carry out daily visual monitoring of water resources and to use other parameters or tools (such as probes equipped with sensors) to supplement this visual surveillance. Such equipment would then improve responsiveness.

The "Cyanobacteria" WG therefore recommends, as an initial approach to this monitoring (see decision tree, Figure 1), daily visual surveillance of the resource by the operator in order to detect the appearance of any cyanobacterial blooms in real time, mainly through a change in the colour of the water. This visual surveillance of resources is a direct approach that should be combined with spot or continuous measurements (by sensors) of several physico-chemical parameters of the water: pH, turbidity and dissolved oxygen as a minimum. For sites known or likely to be vulnerable to cyanobacterial blooms, the "Cyanobacteria" WG also recommends monitoring chlorophyll-a in order to anticipate variations in phytoplankton biomass and/or other pigments more specific to cyanobacteria, such as phycocyanin.

At the first sign of a cyanobacterial bloom (change in colour of the water body, presence of accumulations on the surface, change in odour and/or taste of the water) and/or a rapid and significant variation in at least one of the physico-chemical parameters monitored, the operator should analyse the situation and inform the competent authorities. In addition, the competent authorities could carry out spot sampling and analyses to better determine the extent of the bloom (e.g. over the entire water body surface and throughout the water column) and the impact on the quality of water supplied to the treatment system. The surveillance strategy should be defined locally and be based, where appropriate, on the conclusions of the water safety plan. The operator should then promptly take appropriate measures to adapt its treatment system.

As cyanobacteria are able to move vertically in the water column, the experts recommend, where the design of the water intake allows this, varying the depth at which water is pumped for DW production to avoid drawing water from the layer most contaminated with cyanobacteria. The operator should also ensure that surveillance of the treatment system is tailored to the situation: it may be necessary to screen for toxins at different treatment steps to identify the critical step and optimise the system, particularly if cyanotoxins are found in treated water.

When toxins are detected in treated water, management measures should be proposed, depending on the values measured.

Lastly, if the operator is concerned about any signs of cyanobacterial blooms in the resource and/or operating anomalies in the system (e.g. clogging of filters, significant loss of head), it should adapt the treatment system and notify the ARS. The ARS will then carry out additional analyses (vigilance step) in order to verify whether or not the changes observed are linked to cyanobacterial blooms.

Sanitary control by the ARS

Alongside the surveillance carried out by the operator, sanitary control of the resources by the ARSs should be conducted all year round, at least according to the provisions defined in the amended Ministerial Order of 11 January 2007. As an initial step in this control, the "Cyanobacteria" WG recommends determining chlorophyll-a concentrations and identifying cyanobacteria and then, if any toxigenic genera are identified, counting their cells and assessing their total biovolumes in raw water. It should be remembered that with a potentially toxic genus growing in a lake or river, its populations may be present in proportions that vary over time and sometimes also in space at the scale of the water body, and may consist of individuals with or without the genetic material necessary for cyanotoxin synthesis. The "Cyanobacteria" WG recommends estimating biovolumes of the identified genera of toxigenic cyanobacteria just to serve as indicators of the possible presence of toxins, in the same way as the overall phytoplankton biomass (expressed as chlorophyll-a concentration per unit volume of water).

The recommendation to monitor chlorophyll-a during sanitary control will make it possible to investigate whether a correlation can be established for each water body between the concentration of chlorophyll-a and the biovolume of potentially toxigenic cyanobacteria, with a view to simplifying the decision tree if possible. This could enable this parameter to be used as a monitoring indicator, given that to date it has not been possible to propose a management threshold based on chlorophyll-a for DW. Indeed, a threshold calculated in the same way as for bathing water would be too low and would lead to a state of vigilance being systematically declared.

The experts set a vigilance threshold expressed in total biovolume[12] in the water body of the toxigenic genera, of 0.65 $mm^3.L^{-1}$. This threshold was calculated in relation to the maximum tolerable concentration of MCs. If it is exceeded, the frequency of sanitary control should be increased (once a week) and the treated water should be screened for toxins (total fraction) associated with the potentially toxigenic genera identified (Alert 1). This reinforcement of quality monitoring should be maintained until the biovolume of potentially toxigenic cyanobacteria falls below the threshold of 0.65 $mm^3.L^{-1}$.

Regarding Alert 1, the "Cyanobacteria" WG set threshold values for several cyanotoxins based on the calculation of a guideline value as defined by the WHO, assuming that the proportion of exposure attributable to water was 100%. Therefore, on the basis of the new TRVs developed by ANSES for MC, CYN and STX, the guideline values not to be exceeded for the population of children under six years of age (the most sensitive population) are shown in Table V. The proposed toxin values are for total toxins (intra- and extracellular) and all variants of each toxin. The toxicological data available to date relate only to ingestion.

In the event of a level 1 alert or evidence of cyanotoxins in the treated water, the "Cyanobacteria" WG recommends intensifying monitoring of the treatment system.

For ATX-a and STX cyanotoxins, which can generate acute effects, exceeding the limit of detection for ATX-a and the daily exposure dose corresponding to the acute TRV for STX leads directly to implementation of the management measures planned in the event of Alert 2.

For MCs and CYNs, the guidance values are based on effects observed in rodents during subchronic exposure to cyanotoxins (exposure longer than 14 days). The "Cyanobacteria" WG then recommends that if the guideline values are exceeded, surveillance of toxins in treated water should be continued and the time taken to return to normal (i.e. no toxins detected in treated water) should not exceed cumulative exposure of seven days. This tolerance period will enable the operator to identify the critical step(s) in the process and take appropriate corrective measures before the next sanitary control sample.

If Alert 2 is reached, the "Cyanobacteria" WG recommends switching the resource temporarily while continuing to monitor cyanobacteria counts and cyanotoxin concentrations in the incriminated resource, until the biovolume and toxin concentration fall below the respective threshold values defined in Alert 2. If no other resources are available, the "Cyanobacteria" WG recommends restricting the use of water produced by the incriminated resource and providing the population with bottled water, for example.

[12] Biovolume: cell volume associated with each species or genus of cyanobacteria

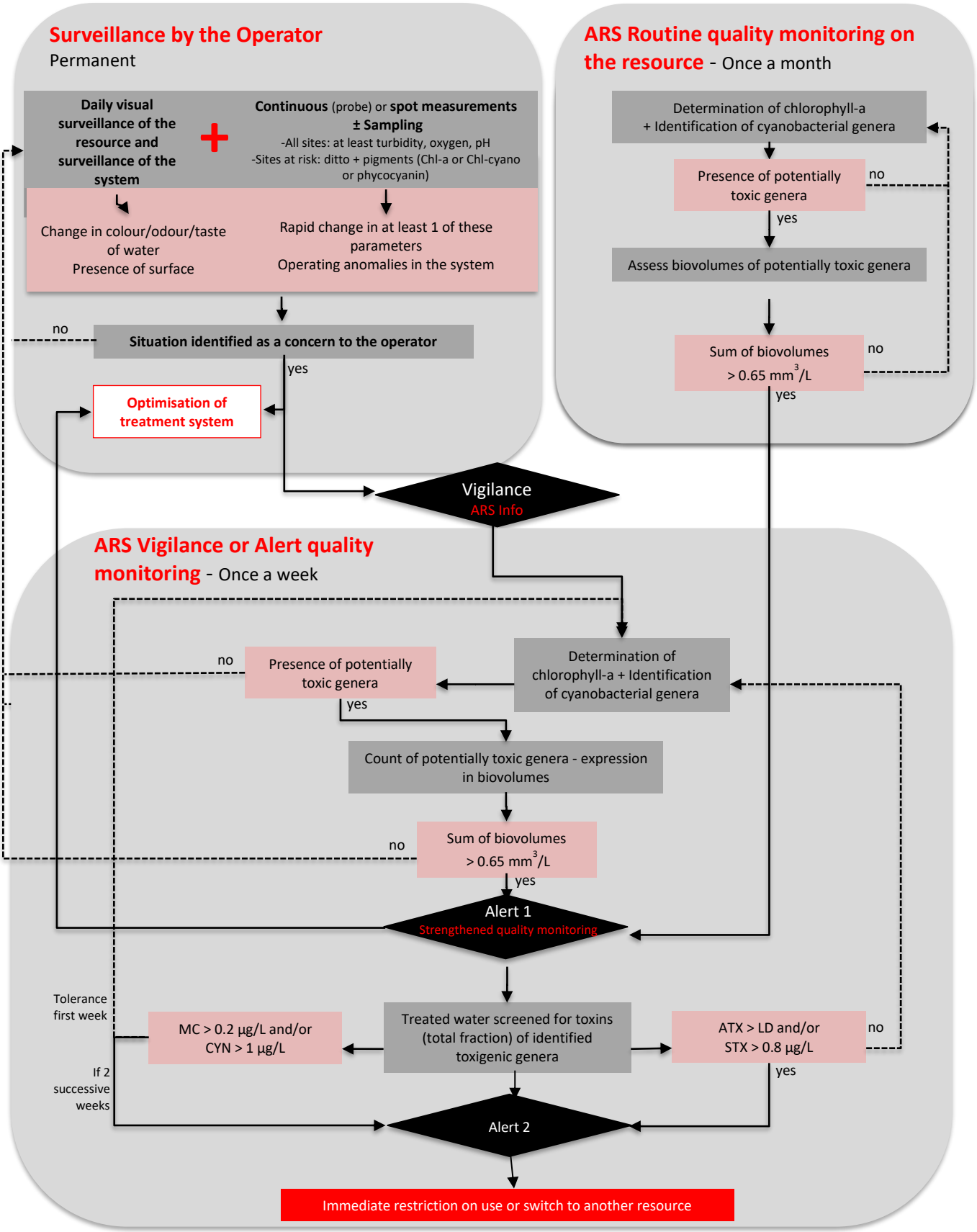

Figure 1: Proposed strategy for surveillance and sanitary control of DW with regard to planktonic cyanobacteria

Benthic cyanobacteria

Given the typology of the water bodies used for DW production and in particular their depth, which is not favourable to the development of benthic cyanobacteria, the "Cyanobacteria" WG does not recommend systematic surveillance of benthic cyanobacteria in resources used for drinking water production. On the other hand, if benthic cyanobacterial blooms are found upstream of DW water intakes, it recommends carrying out ATX analyses in the treated water.

3.12. Monitoring strategy for recreational water and health management

3.12.1. Planktonic cyanobacteria

The implementation of a surveillance programme tailored to recreational water will reduce the risk of exposure of users and professionals to toxins associated with cyanobacteria.

As in the case of water bodies used for DW production, the "Cyanobacteria" WG recommends routine surveillance organised by the site manager and regular sanitary control by the health authority (Figure 2).

In the case of water sports areas, in the absence of regulatory provisions for sanitary control of these sites, any monitoring that needs to be implemented in view of the surveillance results will be the responsibility of the site manager, which could then rely on the provisions proposed below for bathing water surveillance.

3.12.1.1 Bathing water

Surveillance by the manager

As in the case of resources used for DW production, visual checks of water bodies carried out by managers provide the first indicator of cyanobacterial blooms. They can be combined with photosynthetic pigment monitoring using probes.

At the first sign of a cyanobacterial bloom (change in colour of the water body, presence of accumulations on the surface) and/or a rapid variation in the concentrations of the parameters monitored by the probes or in the event of animal mortality (domestic or wildlife), the entity responsible for the bathing site must inform the competent authorities so that they can carry out additional analyses to assess the health risk.

Sanitary control by the ARS

Alongside the surveillance carried out by managers, the "Cyanobacteria" WG recommends visual observation of the water body coupled with determination of chlorophyll-a concentrations, during quality monitoring at the bathing site. If the chlorophyll-a threshold of 10 $\mu g.L^{-1}$ is exceeded, the cyanobacteria present in the water should be identified (Vigilance). If the presence of potentially toxigenic genera is identified, the cyanobacteria should be counted. In this case, the frequency of quality monitoring should be increased (once a week). As in the case of resources used for the production of DW, counting results should be expressed in biovolume.

Alert 1 is triggered if the sum of the biovolumes is greater than 1 $mm^3.L^{-1}$. The toxins likely to be produced by the identified toxigenic cyanobacteria should then be screened for.

When toxins are found, as in the case of resources used to produce DW, the following management measures are recommended by the "Cyanobacteria" WG:

For ATX and STX cyanotoxins, exceeding the detection limit for the first family and 30 $\mu g.L^{-1}$ for the second, should lead to a ban on bathing and information being provided to the public (Alert 2).

For MC and CYN, when the guideline values of 0.3 $\mu g.L^{-1}$ and 42 $\mu g.L^{-1}$ respectively are exceeded, the management measures defined for Alert 2 should be implemented.

In order to limit consumer exposure, the "Cyanobacteria" WG recommends avoiding consumption of fish when Alert 2 is triggered (pending the results of fish analyses for cyanotoxins).

If the thresholds and guideline values are not exceeded, the public should still be informed of the risk of

cyanobacteria, but recreational activities and bathing can be maintained.

3.12.1.2 Water sports areas

There are three main routes of exposure to cyanobacteria during water sports: ingestion, inhalation and through the mucocutaneous barrier. Cyanobacteria and their cyanotoxins can be inhaled through contaminated aerosols generated during water sports such as water skiing. Activities involving occasional immersion of the head (windsurfing, dinghy sailing, canoeing, kayaking or similar) can lead to exposure by ingestion through the mouth or even the nose.

For this reason, during a level 2 alert, the "Cyanobacteria" WG recommends avoiding practising the above-mentioned water sports on or near the sites concerned.

Managers and sports educators who supervise water sports can adapt measures restricting these activities according to the local context and the level of practice of users.

3.12.1.3 General recommendations

Whenever Alert 1 is reached, the "Cyanobacteria" WG recommends putting up signage for the public visiting the sites concerned. In this case, it will be necessary to erect signs near the areas of use, warning of the risks associated with the presence of cyanobacteria (Annex 5), and signs displaying the results of health monitoring and the possible restriction measures. The "Cyanobacteria" WG also recommends that any complaints from bathers and people practising water sports that could be attributable to toxic cyanobacteria (gastric disorders, diarrhoea, itching, etc.) be recorded at lifeguard stations and notified to the ARSs and CAPs.

In general, regardless of the site (bathing areas or water sports areas), whenever a sign informing the public of the presence of cyanobacteria is put up, the "Cyanobacteria" WG recommends disseminating the following advice:

- children should be prevented from playing with clumps of cyanobacteria that have accumulated on the surface, banks, rocks and/or pebbles along water bodies or rivers;
- if suspicious clinical signs should develop (such as gastroenteritis, itching, redness, conjunctivitis, dizziness, impaired senses) as a result of exposure to contaminated water while bathing or engaging in water sports, individuals should take a shower and consult their doctor.

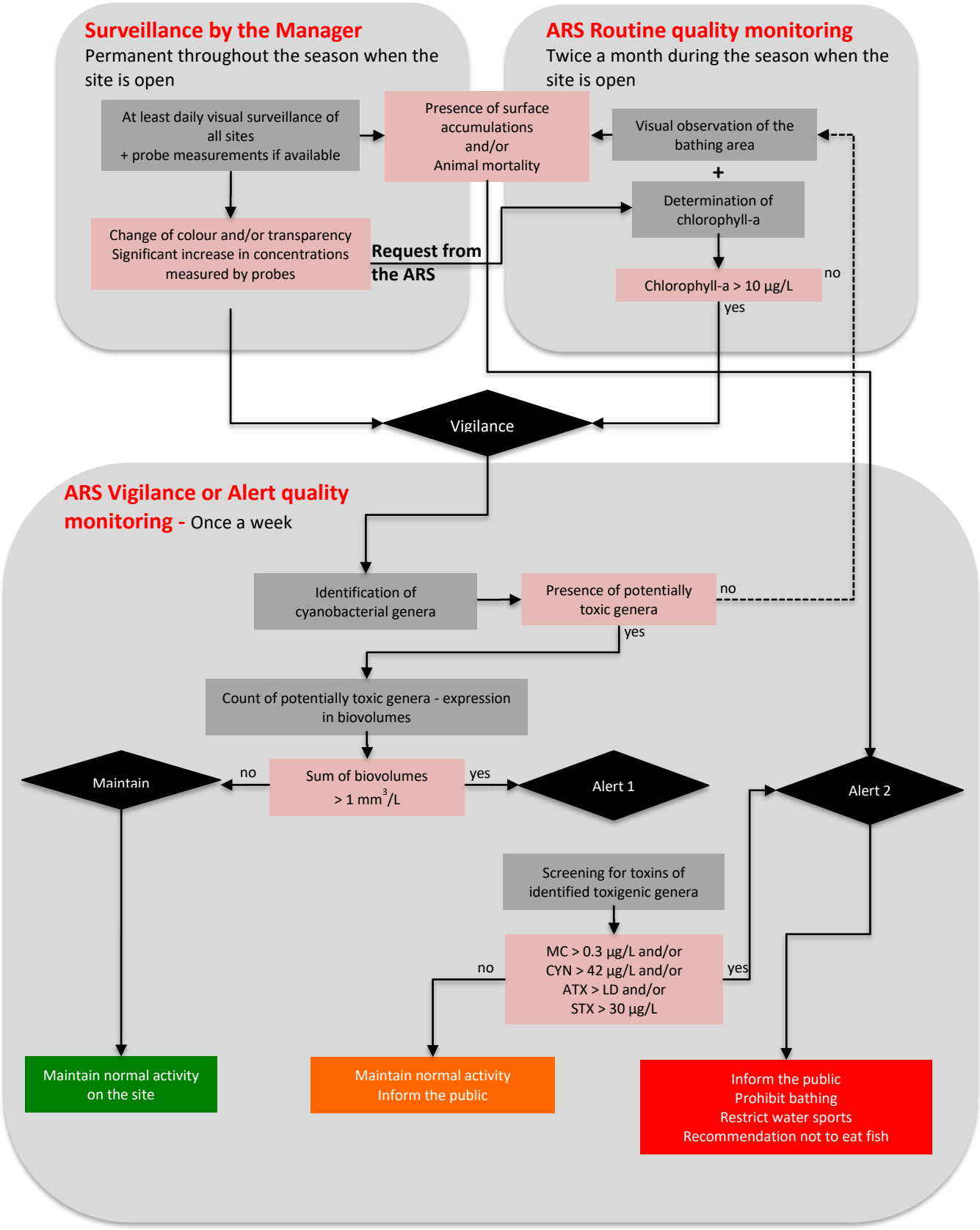

Figure 2: Proposed strategy for surveillance and sanitary control of bathing water with regard to planktonic cyanobacteria

Benthic cyanobacteria

It is more complicated to implement a monitoring plan in rivers, where benthic cyanobacteria are likely to proliferate, because (i) there is great spatio-temporal variability in the development of biofilms and (ii) biofilms produced upstream of the bathing area can spread to this area with the currents. The sampling methods need to be tailored to the local characteristics and their possible changes (for example after rainfall: increase in depth, increase in flow, increase in current speed).

However, it does not appear necessary to carry out systematic monitoring of benthic cyanobacteria in all rivers.

Surveillance by the manager

The sites to be monitored by managers are river areas frequented by the public and/or which have already experienced benthic cyanobacterial blooms. The "Cyanobacteria" WG proposes that managers implement suitable targeted surveillance (Figure 3) during the bathing season (four months during the summer period), on a weekly basis and after any increase in river flow following a period of low water levels, as this is when biofilms are likely to become detached from their substrates and be visible in the form of flocs floating on the surface.

For sites that have already experienced episodes of benthic cyanobacterial blooms, managers should inform the public by posting signs (citizen vigilance should also be encouraged via telephone calls/photos).

For all monitored sites, if the development or accumulation of detached biofilms is observed on the surface (Vigilance), information to the public should be reinforced, by means of posters such as those proposed by the Lozère ARS or the Centre-Val de Loire region ARS (see Annex 5) or by making flyers available in campsites, hotels and tourist offices. The "Cyanobacteria" WG also recommends that managers propose avoiding activities in areas of cyanobacteria growth and accumulation, supervising young children and encouraging dog owners to keep their dogs out of the growth area. Managers should then monitor the situation. If the site concerned is a bathing area, managers should also notify the authority responsible for sanitary control, of the observation of biofilms.

Sanitary control by the ARS

The WG recommends that quality monitoring of bathing areas in rivers be carried out routinely twice a month by the competent health authority, during the four summer months, at all bathing areas that have already shown visible growth of benthic cyanobacteria and if requested by managers.

Declaring a state of vigilance following observations of biofilms as part of routine sanitary control or following a request from the manager will imply a reinforcement of the control previously carried out once a week. The dominance of cyanobacteria in the biofilm samples collected should be checked. If these observations confirm the dominance of cyanobacteria (Alert 1), screening for ATX should be performed. If the toxin is detected (Alert 2), the possibility of adapting the bathing area (restricting the size of the defined area or relocating it) to an area not contaminated by cyanobacteria should be discussed. If no adaptation is possible, bathing should be prohibited.

Alert 2 may also lead to a recommendation not to eat fish, whether caught in or outside these bathing areas.

It should be noted that in some regions, in the absence of any recommendations, bloom situations are currently managed by focusing solely on information for the public. This is particularly the case for

The Tarn River gorges, a place frequently affected by this phenomenon in recent years with dog deaths, and where preventive communication on this hazard has been prioritised (coordinated by the Occitanie ARS – Lozère delegation). Posters and flyers are distributed to the population, especially to pet owners and parents of young children (distribution in campsites, tourist offices, canoe rental organisations), and information boards are placed in areas used to access rivers. There is no ban on fishing or consumption, just recommendations (systematic removal of fish guts and heads).

While communication is a key element in managing these episodes, it is important to assess its effectiveness and deployment, if any, in other *départements*.

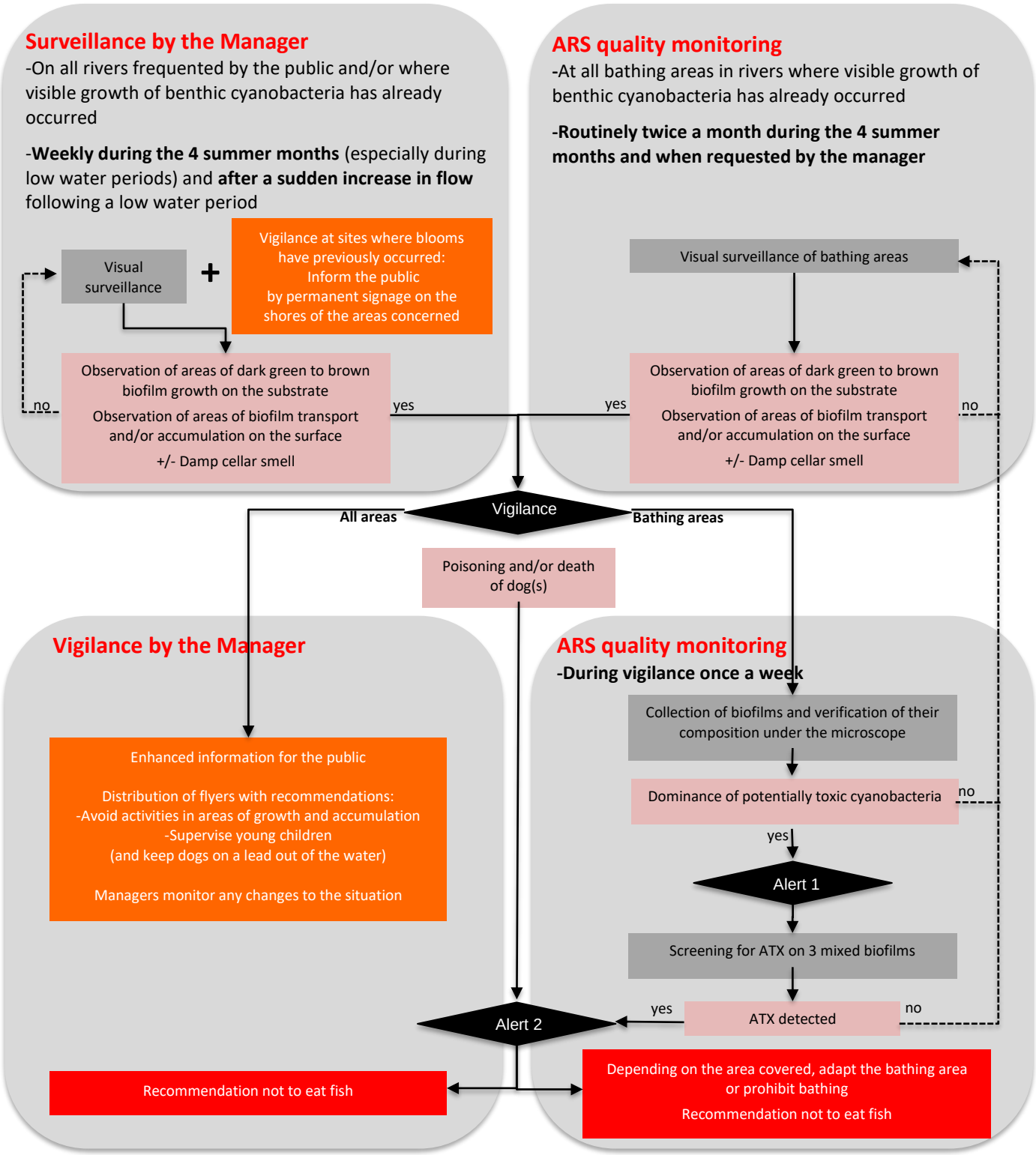

Figure 3: Proposed strategy for surveillance and sanitary control of bathing water with regard to benthic cyanobacteria

3.12.2. General recommendations

In order to harmonise practices and obtain national reference standards for cyanobacteria and their toxins, the "Cyanobacteria" WG also recommends setting up a national reference structure, such as those for pathogenic micro-organisms (like the one for *Legionella*, for example).

Such a structure could be tasked with:

- developing, validating and harmonising sampling protocols for both planktonic and benthic cyanobacteria;
- developing, optimising and validating methods for the analysis of cyanobacteria and their toxins and participating in their validation/standardisation;
- coordinating with ANSES's Nancy Laboratory for Hydrology for the organisation of inter-laboratory tests, confirmation of analysis results by official laboratories, organisation of training sessions;
- responding to any requests for scientific or technical expertise from the ministries;
- maintaining a scientific and technical watch.

4. CONCLUSIONS OF THE EXPERT COMMITTEES ON "WATER" AND "ERCA"

The CESs on "Water" and "ERCA" adopted the conclusions of the "Cyanobacteria" WG.

5. AGENCY'S CONCLUSIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions of the "Cyanobacteria" WG and the CES on "Water" and "ERCA".

The Agency stresses that this expert appraisal work focused exclusively on cyanobacteria found in fresh water (drinking water, recreational water and water intended for professional and recreational fishing). The risks associated with the presence of cyanobacterial blooms in brackish or sea water, or with the presence of cyanotoxins in food supplements or foods of plant origin, were excluded from the scope of the appraisal, as were the risks associated with the ingestion of cyanobacteria and/or cyanotoxins by domestic and wild animals. These issues were in fact incorporated in the Agency's previous work and may, if appropriate, be updated through their inclusion in the Agency's future work programme.

When fresh water has been enriched by anthropogenic inputs of nitrogen and phosphorus, it becomes a particularly favourable breeding ground for planktonic cyanobacteria. Although climate change is also thought to play a role in the duration and intensity of cyanobacterial blooms, human activity is a major contributor, in both the urban and rural environments. ANSES therefore stresses the need to control and reduce nutrient inputs in order to limit this diffuse contamination that mainly affects surface water, as this is the only sustainable solution for protecting and/or restoring aquatic ecosystems from these micro-organisms.

With regard to chemical or even physical treatments, whose use or installation was noted directly in bathing and/or water sports areas in the natural environment, the Agency stresses the need to supervise these practices in view of the risk of toxin release following cell lysis and the associated health risk for humans, as well as the impact on fauna and flora. Furthermore, the use of biocidal products introduced directly into the water resource must comply with Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products, both in terms of approval of the active substance(s) contained in the product and the authorised uses and conditions of use. Their use in water bodies must be compatible with achieving the objectives of the EU Water Framework Directive.

In view of the data provided by local health authorities, operators of water supply facilities and managers of bathing and recreational water, it seems that there is a lack of uniformity in current surveillance and

control practices in France. The Agency emphasises the need to harmonise these practices – especially with regard to surveillance and monitoring methods – through the establishment of national standards. To this end, a national structure bringing together several laboratories competent in the area of cyanobacteria and cyanotoxins, including those of the Agency, would help develop and validate both sampling protocols and cyanotoxin analytical methods. The Agency reiterates that regardless of the analytical method used, results concerning cyanobacteria and cyanotoxins must be reported under accreditation like any other parameter for the quality monitoring of drinking water or bathing water. Indeed, ANSES encourages the certification of ELISA kits on the basis of a third-party validation standard applicable to water, since this latter remains to be developed. This recommendation also applies to screening for cyanotoxins in fish flesh.

In this respect, the Agency notes the lack of knowledge on the contamination and elimination kinetics of cyanotoxins by fish. The Agency therefore reiterates its recommendation to acquire data on this subject and to obtain French data on the contamination of freshwater fish by cyanotoxins, in order to estimate the dietary exposure of consumers. Specific consumption data would enable these estimates to be refined in a more realistic way than on the basis of national consumption data from INCA surveys.

Furthermore, the Agency insists on the need to train resource operators and water body managers in the recognition of planktonic and benthic cyanobacterial blooms in the field, as well as to reinforce the training of laboratory staff in the identification, by microscopy, of planktonic and benthic cyanobacteria genera in general and potentially toxic genera in particular.

Lastly, the decision trees proposed for the surveillance of DW or recreational water are derived from ANSES's updating of the toxicity reference values for microcystin-LR, cylindrospermopsin and saxitoxin. For microcystin-LR, this value has been divided by a factor of 40 compared to the one formulated by the WHO in 1998; this is justified by taking into account new toxicity data in animals and a more sensitive critical effect (reprotoxicity instead of hepatotoxicity).

Roger GENET

KEYWORDS

Cyanobacteria, cyanotoxins, freshwater fish, microcystins, cylindrospermopsin, saxitoxins, anatoxin-a drinking water, recreational water, guideline values, analytical method, monitoring

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ANNEX 1: RECOMMENDED BIOVOLUMES ACCORDING TO THE GENUS OF CYANOBACTERIA

Genus name	SISE code	Cell biovolume in µm³
Anabaena	CYANO01	99.0
Anabaenopsis	CYANO02	125.0
Aphanizomenon	CYANO03	72.0
Aphanocapsa	CYANO04	2.0
Aphanothece	CYANO05	10.0
Arthrospira		96.0
Calothrix	CYANO06	215.0
Chroococcus	CYANO07	122.0
Chrysosporum		133.0
Coelomoron	CYANO08	8.1
Coelosphaerium	CYANO09	4.0
Cuspidothrix		95.0
Cyanobium		43.0
Cyanocatena	CYANO45	0.4
Cyanodictyon	CYANO41	2.0
Cyanogranis	CYANO48	1.0
Cyanonephron		2.0
Cylindrospermopsis	CYANO10	70.2
Cylindrospermum	CYANO11	65.7
Dolichospermum		290.0
Eucapsis		14.0
Fischerella	CYANO12	261.3
Geitlerinema	CYANO51	19.7
Glaucospira		36.0
Gloeocapsa		245.0
Gloeotrichia	CYANO13	287.6
Gomphosphaeria	CYANO14	11.0
Hapalosiphon	CYANO15	236.5
Homoeothrix	CYANO40	16.3
Jaaginema		18.8
Komvophoron	CYANO52	80.0
Lemmermanniella	CYANO16	2.2
Leptolyngbya	CYANO42	6.3
Limnothrix	CYANO17	31.0
Lyngbya	CYANO18	56.0
Merismopedia	CYANO19	13.0
Microcoleus	CYANO20	263.0

Microcystis	CYANO21	50.0
Nodularia	CYANO22	170.7
Nostoc	CYANO23	50.1
Oscillatoria	CYANO24	410.0
Pannus	CYANO47	2.9
Phormidium	CYANO25	177.0
Planktolyngbya	CYANO26	4.0
Planktothrix	CYANO39	52.0
Pseudanabaena	CYANO27	43.0
Radiocystis	CYANO43	30.8
Raphidiopsis	CYANO28	70.2
Rhabdoderma	CYANO29	16.0
Rhabdogloea		82.0
Rivularia	CYANO49	171.6
Romeria	CYANO46	4.0
Schizothrix	CYANO30	8.8
Scytonema	CYANO31	1,565.0
Snowella	CYANO34	7.0
Sphaerospermopsis	CYANO50	79.7
Spirulina	CYANO32	177.0
Symploca	CYANO33	55.6
Synechococcus	CYANO35	55.0
Synechocystis	CYANO44	4.0
Tapinothrix		16.3
Trichodesmium	CYANO36	113.0
Umezakia	CYANO37	226.0
Woronichinia	CYANO38	15.0

ANNEX 2: KEYS TO THE VISUAL DETERMINATION OF A PLANKTONIC CYANOBACTERIAL BLOOM

CARACTERES GENERAUX DES CYANOBACTERIES	
Couleur	Les cyanobactéries sont appelées « algues bleues », elles sont généralement bleues - vertes, cependant certaines sont de couleur rouge.
Taille des particules	Les particules peuvent être à peine perceptibles de près, leur agglomération peut mesurer seulement quelques millimètres voire moins.
Odeur	Des odeurs de gazon fraîchement coupé ou d'ordures peuvent accompagner un fort développement (efflorescence).
Zone de prolifération	Les proliférations peuvent s'étendre sur tout le plan d'eau ou être très localisées (anses calmes, orientation aux vents dominants).
Localisation dans la colonne d'eau	Les proliférations de cyanobactéries peuvent occuper la surface de l'eau mais aussi la colonne d'eau sur 1 ou plusieurs mètres de profondeur.
Apparence	Les cyanobactéries peuvent présenter différents aspects notamment en fonction du stade de développement : × simples particules dispersées pouvant rendre l'eau turbide, × masse importante dans la colonne d'eau - « purée de pois », × film, traînées de surface ressemblant à un déversement de peinture, × écume colorée.

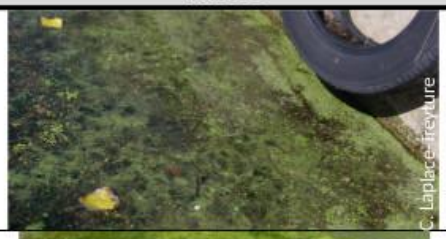
RAPPEL : EN CAS DE PROLIFÉRATION DE CYANOBACTÉRIES, LE RÉSEAU DE SURVEILLANCE DOIT :

- **remplir la fiche d'observations,**
- **contacter le SMGBL, pour éventuellement effectuer** un prélèvement.

Version 3.0

CLE DE DETERMINATION		
1. Observer de près le phénomène et vérifier s'il comporte des « particules », des « éléments » flottants ou en suspension dans la colonne d'eau.	Absence de particules.	Ce n'est pas une prolifération de cyanobactéries
	Présences de particules, d'éléments flottants.	Aller au 2
2. Prendre des gants en latex, Passez votre main dans la zone de prolifération en écartant légèrement les doigts, Laissez couler l'eau.	Si de longues masses fibreuses plus ou moins rigides pendent à vos doigts.  	Aller au 3
	S'il ne reste rien ou juste quelques morceaux visqueux collés à vos gants.  	Aller au 4
3. La masse garde-t-elle sa forme hors de l'eau avec des éléments de formes différentes ?	Oui, avec des feuilles, des tiges et des racines.	**Plantes aquatiques - A**
	Non, filaments minces de formes identiques.	**Algues filamenteuses - B**
4. Une partie de la prolifération est-elle fixée au fond au moins partiellement ?	Oui	Aller au 5
	Non	Aller au 6
5. Quelle est la couleur et l'allure de la prolifération ?	Masse floconneuse de couleur jaunâtre à brunâtre.	**Probablement un genre de diatomée : *Didymosphenia* - C**
	Tapis assez fins, plus ou moins visqueux de couleur verte à brune.	**Périphyton - D**
6. Quelle est la couleur et l'aspect de la prolifération ?	Particules flottantes d'apparence poudreuse, de couleur jaunâtre.	**Pollens ou spores - E**
	Particules brunes en suspension dans la colonne d'eau.	**Diatomées planctoniques - F**
	Mousse en surface de couleur beigeâtre.	**Mousses lacustres - G**
	Particules de couleur essentiellement bleu-vert ou rouge, sous forme d'amas, de billes dans la colonne d'eau et/ou en surface.	**Cyanobactéries - H**

Version 3.0

DESCRIPTION DES DIFFERENTS TYPES DE PROLIFERATIONS				
Type	**Noms**	**Couleurs**	**Caractéristiques**	**Photos**
A	Plantes aquatiques Macrophytes vasculaires	Vert, brun	Composées de feuilles, tiges, racines, thalles dont tout ou partie peut flotter. *Aspect de particules ou de tapis flottants.*	
B	Algues filamenteuses Macrophytes non vasculaires	Vert	Filaments minces, très souples qui flottent souvent à la surface de l'eau ou accrochés à un support. *Parfois aspect de cheveux mêlés.*	
C	*Didymosphenia* Microalgues de la famille des diatomées	Entre blanc et jaune brunâtre	Filaments formant des amas visibles à l'œil nu. *Humide la texture s'apparente à de la laine mouillée et en séchant à du parchemin.*	
D	Périphyton Algues benthiques	Verdâtre, brun	Tapis visqueux ayant une apparence plus ou moins mousseuse. *Lorsqu'il est mort et séché, il peut avoir l'aspect du papier.*	
E	Pollens ou spores	Jaunâtre	Particules flottantes d'apparence poudreuse.	
F	Diatomées Microalgues siliceuses unicellulaires planctoniques	Brunâtre	Particules en suspension donnant une eau turbide.	
G	Les mousses lacustres	Beigeâtre, blanchâtre	Elles peuvent accompagner une efflorescence de cyanobactéries ou se produire à la suite d'un épisode de vent. Elles sont remarquables lorsqu'elles s'accumulent en bordure.	

Version 3.0

H	EXEMPLES D'EFFLORESCENCE DE CYANOBACTERIES OBSERVES	
Simples particules dispersées pouvant rendre l'eau turbide		
Masse importante dans la colonne d'eau type « Purée de pois »		
Film, trainées en surface type « Déversement de peinture »		
Ecume colorées en bleu-vert ou brunâtres		

CONTACTS & COMPLÉMENTS D'INFORMATION

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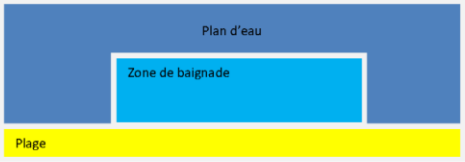
Version 3.0

ANNEX 3: EXAMPLE OF A CYANOBACTERIA INFORMATION SHEET FOR BATHING AND/OR WATER SPORTS AREAS (BASED ON A DOCUMENT FROM THE SMGBL AND IRSTEA)

Fiche de renseignements : cyanobactéries et analyse de toxines dans des zones de baignade et/ou d'activités nautiques

ZONE DE BAIGNADE		OBSERVATEUR	
Zone de baignade		Nom prénom	
Plan d'eau/cours d'eau		Téléphone	
Commune		Structure	

CONTEXTE			
Date et heure d'observation	Le ..... / ..... /..... à ..... h		
Température de l'air (°C)	(°C)	Température de l'eau (°C)	(°C)
Conditions météorologiques	☐Soleil ☐Pluie ☐Couvert ☐ Brouillard ☐Autre :		
Etat de la surface de l'eau	☐ Lisse ☐ Faiblement agitée ☐ Agitée ☐ Très agitée		
Vent	☐Nul ☐Faible ☐Moyen ☐ Fort		
Direction du vent	..... ☐ Vers la zone de baignade ☐ Vers l'extérieur de la zone de baignade		

PROLIFERATION DE CYANOBACTERIES	
Localisation	☐dans la zone de baignade ☐à proximité de la zone de baignade ☐éloignée de la zone de baignade Précisions :..... ....
Schéma *Indiquez la localisation de la prolifération et du(es) prélèvement(s) si réalisé(s)*	
Etendue de la prolifération dans la zone de baignade	☐ Inconnue ☐ Généralisée (environ 75%) ☐ Grande (50 à 75 %) ☐ Limitée (moins de 50%) ☐ Très limitée (inférieur à 25%)

OBSERVATIONS			
Photographies	☐ Oui ☐ Non	Nombre	
Couleur de l'eau si inhabituelle	☐ Violet ☐ Bleu ☐ Vert ☐ Jaune ☐ Orange ☐ Rouge		
Intensité de la coloration	☐ Légèrement coloré ☐ Moyennement coloré ☐ Fortement coloré		
Apparence et intensité	☐ La densité est faible : particules réparties de façon clairsemée dans la colonne d'eau. *(eau anormalement trouble, particules flottant entre deux eaux, traînées clairsemées en surface)* ☐ La densité est moyenne à élevée : particules distribuées dans la colonne d'eau. *(soupe au brocoli plus ou moins consistante, purée de pois, agrégats ou amas nombreux ou rapprochés)* ☐ La densité est très élevée : particules concentrées à la surface de l'eau (écume). *(films ou traînées opaques à la surface, déversement de peinture, dépôt près du rivage)*		
Présence d'écume	☐ Oui ☐ Non	Dépôts sur le rivage	☐ Oui ☐ Non
Odeur	☐ Oui ☐ Non		
Commentaires			

PRELEVEMENT			
Nom prénom du préleveur		Date et heure de prélèvement	Le ..... / ..... /..... à ..... h .....
Type de prélèvement	☐ Cyanobactéries ☐ Toxines	Nombre de prélèvements	
Fixation du prélèvement de cyanobactéries	☐ Non ☐ au lugol ☐ au glutaraldéhyde ☐ autre :		
Transport au laboratoire	☐Par la collectivité ☐ Par le laboratoire		

Version 1.0

ANNEX 4: PROTOCOL FOR COUNTING PLANKTONIC CYANOBACTERIA

Quantification under the microscope is carried out on phytoplankton or benthic samples fixed with Lugol's solution.

It is then recommended to count at least 100 individuals to obtain a satisfactory result with an error of the order of +/-10%. Counting is performed on a sample volume of 1 to 25 mL maximum, depending on the concentration. The sedimentation time then varies from 25 minutes (for 1 mL) to 18 hours (for 25 mL) at a rate of 4 hours/cm column height. Typical volumes used are 1 to 3 mL for bathing water.

For DW, since the concentration of cyanobacteria is expected to be very low, a volume of 1 litre should be filtered on a 3 µm polycarbonate membrane of medium porosity (diameter 2.5 cm). The algae retained by the filter must be re-suspended in one millilitre of demineralised water directly in the sedimentation chamber by gently rubbing its surface with a gloved hand.

It is important to systematically start with an initial observation at low magnification (100x) of one millilitre of the sample, deposited at the bottom of the sedimentation chamber. This gives an idea of the concentration of the sample, and makes it possible to check the random distribution, observe whether algae are floating on the surface and identify the main genera present. If the number of individuals present appears to be sufficient to achieve the counting of 100 individuals and the distribution appears to be random, then the sample can be counted directly in a millilitre. If this is not the case, a larger sedimentation volume must be produced.

If cyanobacteria are floating on the surface, the gas vesicles should then be burst. This step is performed using a 50-60 mL syringe into which a fraction of the homogenised sample is placed. The end of the syringe should be blocked with a finger and then the plunger tapped vigorously 2 to 3 times on a flat surface. The sample is then transferred to a bottle, homogenised again and a millilitre is removed for observation in the sedimentation chamber. The buoyancy of cyanobacteria is again monitored. If no significant cyanobacteria are floating, then the sample can be counted in a millilitre or more if necessary.

Counting is then carried out in two phases. First, large individuals are counted with low magnification (100x) on the entire chamber or a smaller area (several transects) if the individuals are too abundant. A random field or transect count at higher magnification (400x or even 600x) is then carried out to count all other smaller cyanobacteria. When using fields, a minimum of 20 fields must be counted. If transects are used, a minimum of one transect count is required.

During these two successive counting phases, a total of at least 100 individuals must be counted, in which the number of cells is estimated (case with colonies and filaments) or determined (unicellular) following the counting rules defined in the NF EN 15 204 Standard. The result is then expressed as cells per millilitre (cell/mL).

To overcome the problem of cell size variability, the experts then recommend converting counts into biovolume (biomass): see Annexe 1. The result of the count then enables the total quantity of cyanobacteria present in the phytoplankton sample to be assessed, while identifying the presence and biomass of potentially cyanotoxin-producing genera.

ANNEX 5: EXAMPLE OF A SIGN PROPOSED BY THE ARSS