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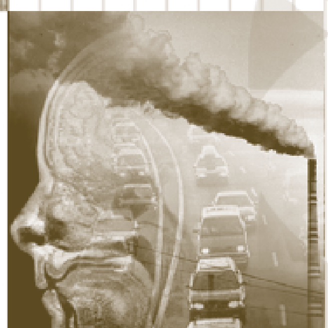

Cyanobacteria in water for human consumption
GUIDELINES FOR RISK MANAGEMENT

ISSN 1123-3117

English translation by L. Lucentini, E. Ferretti, V. Fuscoletti and F. Nigro Di Gregorio

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English translation by
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Dipartimento di Ambiente e Connessa Prevenzione Primaria

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English translation edited by Luca Lucentini, Emanuele Ferretti, Valentina Fuscoletti and Federica Nigro Di Gregorio
2013, viii, 64 p. Rapporti ISTISAN 13/35

This report represents the synthetic transposition of the state of knowledge from research and technological development in the field of cyanobacteria, reported in detail in the Italian volume *Rapporti ISTISAN* 11/35 Pt. 1. The guidelines consist of operating and management recommendations addressed to water supply systems and aimed at improving the quality of the response to emerging issues of cyanobacteria, ensuring optimization of interventions in health protection and rational use of resources. They are designed as a tool for health and environmental monitoring routine, and describe the structuring and implementation of a system based on an Alert Level Framework and the principles of Water Safety Plans for the risk management extended to the drinking water supply chain, from the reservoir to control user points. There is also a specific discussion of strategies for emergency response and contingency plans, and risk mitigation measures, including prevention and treatment for removal of cyanobacteria and toxins. Finally, the principles and methods to ensure adequate information and communication between stakeholders and consumers, and information, criteria and methodologies needed to implement a system of syndromic surveillance (epidemiological observatory) are also reported.
Keywords: Cyanobacteria; Cyanotoxins; Waters for human consumption; Drinking waters; Risk assessment; Risk management

Istituto Superiore di Sanità
Cianobatteri in acque destinate a consumo umano. Linee guida per la gestione del rischio.
Traduzione inglese a cura di Luca Lucentini, Emanuele Ferretti, Valentina Fuscoletti e Federica Nigro Di Gregorio
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Questo volume rappresenta una trasposizione sintetica e pratica dello stato delle conoscenze dal settore della ricerca e dello sviluppo tecnologico nel campo dei cianobatteri (riportato in maniera più approfondita nel volume italiano *Rapporti ISTISAN* 11/35 Pt. 1). Le linee guida consistono di istruzioni operative e raccomandazioni gestionali rivolte ai sistemi idrici per migliorare la qualità della risposta alle problematiche dei cianobatteri garantendo l'ottimizzazione degli interventi a protezione della salute e la razionalizzazione dell'utilizzo delle risorse. Concepite come strumento per la vigilanza sanitaria e ambientale di routine, le linee guida descrivono la strutturazione e implementazione di un sistema basato sull'approccio *Alert Level Framework* e sui principi dei *Water Safety Plan* per la gestione del rischio esteso all'intera filiera idro-potabile, dal controllo dell'invaso ai punti di utenza. Specifica trattazione trovano le strategie di risposta alle emergenze e le misure di mitigazione del rischio, comprendenti prevenzione e trattamenti di rimozione di cianobatteri e tossine, e dei piani di emergenza. Sono, infine, riportati i principi e gli strumenti per garantire un'adeguata informazione e comunicazione tra le parti interessate e i consumatori e le informazioni, i criteri e le metodologie necessarie all'implementazione di un sistema di sorveglianza sindromica (osservatorio epidemiologico).
Parole chiave: Cianobatteri; Cianotossine; Acque destinate a consumo umano; Acque potabili; Valutazione del rischio; Gestione del rischio

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5. Risk information and communication

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ACRONYMS

ADDA	3-Amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-DecaDienoic Acid
ALF	Alert Level Framework
ANA-a	Anatoxin-a
API	Atmospheric Pressure Ionization
ATP	Adenosine Triphosphate
BMAA	Beta-Methylamino-L-Alanine
CCM	*Centro Nazionale per la prevenzione e il Controllo delle Malattie*
CID	Collision Induced Dissociation
CIMF	Cyanobacterial Incident Management Framework
CV	Coefficient of Variation
CYN	Cylindrospermopsin
DAD	Diode Array Detector
DOC	Dissolved Organic Carbon
DP	Declustering Potential
DRP	Dissolved Reactive Phosphorus
EDTA	EthyleneDiamineTetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
EP	Entrance Potential
FEP	Fluorinated Ethylene Propylene
FLD	Fluorescence Detector
FP	Focusing Potential
FRP	Filtred Reactive Phosphorus
GAC	Granular Activated Carbon
GC	Gas Cromatography
GPS	Global Positioning System
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
i.d.	Inner diameter
IARC	International Agency for Research on Cancer
IPD	Individual Protection Devices
LC	Liquid Chromatography
LLE	Liquid/Liquid Extraction
LOD	Limit of Detection
LPS	Lipopolysaccharides
MAC	Maximum Acceptable Concentrations
MC	Microcystins
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
NOAEL	No Observed Adverse Effect Level
NOD	Nodularin
NOM	Natural Organic Matter
NRPS	Non-Ribosomal Peptide Synthetase
NTU	Nephelometric Turbidity Unit
PAC	Powdered Activated Carbon
PAR	Photosynthetically Active Radiation
PKS	Polyketide Synthase
POC	Particolate Organic Carbon
PTFE	Polytetrafluoroethylene
Q-TOF	Quadrupole Time Of Flight
RF	Response Factor

RP	Reactive Phosphorus
SD	Standard Deviation
SPE	Solid Phase Extraction
SRP	Soluble Reactive Phosphorus
TC	Total Carbon
TDI	Tolerable Daily Intake
TEF	Toxicity Equivalent Factor
TIC	Total Inorganic Carbon
TIS	Turbo Ion Spray
TLC	Thin Layer Chromatography
TOC	Total Organic Carbon
TP	Total Phosphorus
$\mathbf{t_r}$	Retention time
UPLC	Ultra Performance Liquid Chromatography
UV	Ultraviolet Light
VOC	Volatile Organic Compound
WHO	World Health Organization
WSP	Water Safety Plan

1. INTERNATIONAL STRATEGIES: WATER SAFETY PLAN AND ALERT LEVEL FRAMEWORK

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A critical analysis of literature, the comparison among the strategies developed at international level, the experiences discussed within the working group agree on the definition of a risk management system for cyanotoxins monitoring in water for human consumption structured as follows:

– a risk assessment preventive approach and "multiple barrier" controls based on Water Safety Plan (WSP) principles of the World Health Organization (WHO) (1-4);
– the respect, according to the points of compliance established in the Legislative Decree 31/2001 (art. 6), of the maximum acceptable value for cyanotoxins, currently fixed for MC-LR at 1.0 μg/L; value that has to be applied, according to the maximum precautionary principle, to the sum of microcystins concentrations (see volume *Rapporti ISTISAN* 11/35 Pt. 1 section 3.3).

Based on this assumption, the risk management integrated system to be applied at national level, which follows the Alert Level Framework (ALF) model, is reported in section 2.3. Together with the emergency management plans (see section 3), it represents the main part of the guidelines and provides the decision-making and implementing criteria for the prevention and management stage concerning toxic phenomena related to cyanobacterial blooms in water for human consumption.

Section 1.1. introduces WSP general concepts and the principles regarding their implementation during cyanotoxins monitoring.

1.1. Application of WSP criteria to cyanotoxins risk

1.1.1. WSP for risk assessment and management in the drinking water supply chain

During the last years, the limitations of quality control systems related to water for human consumption, until now characterized by a surveillance over more or less defined segments of the catchment → treatments → distribution → users cycle and/or by a random monitoring on distributed waters, have been substantially redefined. The development of risk analysis knowledge has, indeed, definitely moved the interest towards the creation of a global risk management system involving the entire water supply chain, from catchment to the final user point.

This is the approach included in the WSPs recently introduced by the WHO during the review of the guidelines concerning the drinking water quality (1) and strengthened in the following editions until the most recent of 2011 (3). This approach has been implemented at regulatory level in several Countries of the European area and it has been proposed for a possible introduction in the review of Directive 98/83/EC concerning the quality of water for human consumption.

The WSPs model, extremely straightforward in its general aspects, is aimed to drastically reduce catched waters contamination chances, to diminish or eliminate chemical and microbiological risk factors through water treatments properly designed, carried out and controlled and, eventually, to prevent possible recontaminations during water storage stage and distribution to the final user point.

The strategy presents a high flexibility and it can be applied to any production and distribution system regardless of its nature, legal form, policy, size and complexity.

The principles contained in the WSPs, and synthetically reported in Table 1, can be considered as a reassessment and reorganization of several criteria and management procedures that, until now, have presided over the/governed/lead to the production and distribution of waters with an adequate quality for human consumption, especially when based on quality assurance systems equivalent to ISO 9001:2001; among them, it is the multi barrier control system based on an integrated process to prevent microbiological contamination of water. At the same time, there have appeared crucial elements of risk analysis and management borrowed from other productive sectors and, mainly, from the HACCP system (*Hazard Analysis and Critical Control Points*), compulsory for the food industry and standardized at regulatory level (5).

Table 1. Synthetic representation of WSPs principles

Plan stage	Purpose
Creation of a multidisciplinary team with identification of roles and responsibilities	▪ To establish the risks related to each single component/stage of the water system. ▪ To evaluate the system effectiveness in granting appropriate hygiene and sanitary quality standards.
Water system description	▪ To represent the system and all its components/stages in detail (flow chart): catchment area, cathcment, treatments, storage and distribution network, internal distribution systems. ▪ To identify users segments and uses of distributed waters.
Risks analysis and identification of risk priorities	▪ To identify potential factors of biological, physical and chemical risk related to different elements of the system and the possible events that can cause a health risk for final users. ▪ To establish a risk priority scale based on potential effects and likelihood of occurrence, as basis of each decision-making process.
Definition and validation of adequate measures for monitoring risks	▪ To identify and verify actions so as to monitor each significant risk, through physical barriers or appropriate activities to prevent, eliminate or reduce the likelihood of occurrence or mitigate consequences.
Control and monitoring measures	▪ To carry out, on a systematic basis, a series of process and products controls so as to ensure the effectiveness of the system in taking the risk under control: each control measure must be planned in terms of implementation procedures, safety limits and corrective actions to be taken in case of significant deviations from those limits.
Plan testing	▪ To evaluate the overall effectiveness of plan in granting water compliance – at user point – to hygiene and sanitary quality standards.
Papers and review	▪ To ensure and document, over time, plan functioning effectiveness, based on the results obtained or following to the occurrence of accidents or emergencies.

Due to its nature, a WSP should be developed for each specific water system. Several difficulties in plan designing and implementation stage, can be especially observed in small size water supply systems (Small Water Supplies, SWS) which represent a significant part of the Italian aqueduct system and that also find a specific place in WSPs application manuals (3, 6).

1.1.2. WSP for cyanotoxins monitoring

Cyanobacteria blooms can occur in undamaged natural environments and, more frequently, in water bodies affected by human interferences which directly or indirectly favour algal development. In many cases, this is encouraged by the introduction of nutrients with consequent eutrophication due to agricultural, livestock activities or wastewater, or, in other circumstances, by the change in river course due to the creation of reservoirs for water catchment which increase the retention time and the exposure of the water body to the sunlight (7). Even climate change plays its role in the expansion of algal bloom phenomena related to cyanobacteria. It not only influences temperatures, but it can also entail drastic changes in the hydrodynamic regime of reservoirs, as in the case of shallows rapidly followed by flood flows freeing the nutrients tied up in the sediments (8-10).

The massive development of toxic cyanobacteria frequently occurs in reservoirs previously not affected by proliferation phenomena; on the other hand the reoccurrence of blooming phenomena in already affected reservoirs, has to be considered as normal. In fact, in this last case, populations of cyanobacteria, once installed, persist in the aquatic environment and tend to proliferate in favourable environmental conditions (see section 1.1 of *Rapporti ISTISAN* 11/35 Pt. 1). The likelihood to interrupt the occurrence of these phenomena, over time, is related to complex long term ecological recovery measures such as the monitoring of nutrients introduction, the limitation of sedimentation activity or sediments removal. The scope of these interventions, the discussion of which goes beyond the purpose of these guidelines, entails the involvement of many functions within the global management of internal water, environmental policy, resources development and allocation strategies.

Figure 1 represents a series of preventive interventions and monitoring measures that can be realized in the water body and in the drinking water supply chain so as to eliminate or reduce the possible occurrence of cyanotoxins in water for human consumption; the scheme gives the idea of how the measures have been divided – seen as multiple barrier control in the different stages of the chain –, of the nature of different measures, also in terms of interventions space and time extension and, at the same time, of the need to merge the different actions in a sole integrated and global prevention and control strategy based on WSPs and WHO principles (1-3).

The structuring of a cyanotoxins monitoring based on WSPs approach, whose main elements have been previously recalled, offers some crucial advantages that can be summarized as follows:

- The preventive approach allows to reduce the exposure related to possible overcoming of toxins levels in the distributed water that, in the event of monitoring of distributed water, could be backwardly noticed.
- The preventive measures adopted for cyanotoxins risk are effective for water protection compared to several other risk factors, for example the monitoring of livestock waste prevent other problems related to the eutrophication as well as the spreading of oro-fecal transmission disease agents and protozoa (e.g. *Giardia, Cryptosporidium*).
- Similarly, treatment measures adopted to mitigate risks due to cyanotoxins (e.g. activated charcoal filtration) contribute to the monitoring of other critical parameters such as disinfection by-products and trihalomethanes which tend to gather due to the higher concentration of organic substance in the water caused by the algal mass.

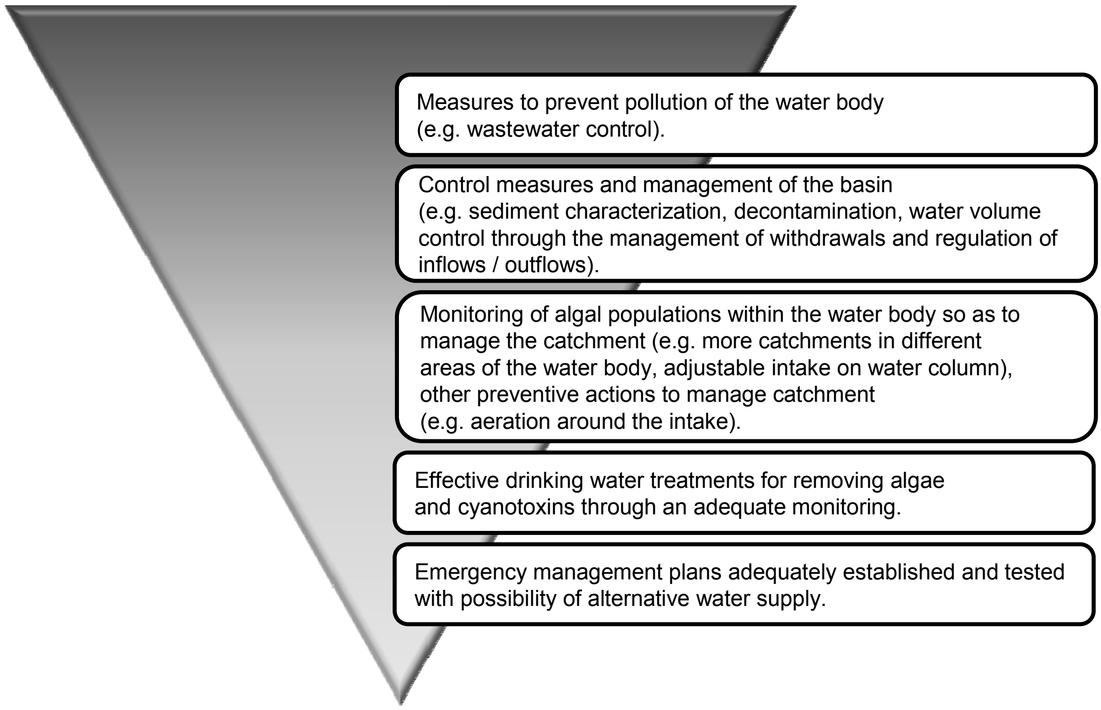

Figure 1. Preventive interventions and monitoring measures in the water body and the drinking water supply chain so as to eliminate or reduce the possible occurrence of cyanotoxins in water for human consumption

1.2. ALF approach for cyanotoxins monitoring

From the healthcare point of view, the main consequence of the events related to cyanobacteria proliferations, is associated to water drinking use. The risk is determined by the possible presence in the water of toxic metabolytes produced by phytoplanktonic organisms. Cyanotoxins, in fact, which are found in significant concentrations under intra-cellular and/or dissolved form, in water for human consumption, if not efficiently removed from the drinking water treatments chain, could persist reaching the final consumer and, if in concentrations exceeding the safety levels, could represent a risk factor for water consumption.

From the surveillance point of view, cyanotoxins represent, in each regard, chemical risk factors. However, the systematic determination of these lasts in the water, contrary to what happen with other parameters which are normally subject to monitoring, is not regularly carried out. Cyanotoxins are not expressly included among the parameters to be monitored in compliance with Directive 98/83/EC and with its implementation at national level, Legislative Decree 31/2001 and amendments. On the other hand, based on specific risk assessments, some Countries have judged useful to establish a cyanotoxins monitoring obligation within the regulation on drinking water quality (see section 2.1.1 of *Rapporti ISTISAN* 11/35 Pt. 1).

In general terms, being the cyanobacteria proliferation generally confined to limited time intervals, the presence of toxins in potentially significant concentrations for human health persists just for short-term periods and, therefore, a periodical control during the entire year could be inappropriate from the point of view of resources allocation. To this, it can be added that within the blooming periods, cyanotoxins levels can significantly change in few days, with the consequent need of a close surveillance whereas a standard continuous control over the year should necessarily envisage prolonged time intervals between samplings and could not be able to identify health risk conditions. Toxins monitoring methods for confirmation purposes are, at present, not available for the overall territory and demand costly instrumental and human resources.

According to this, there have been implemented several monitoring and risk management strategies based on an integrated surveillance of the water body and the drinking water chain, adjusted on existing risk levels within the raw waters and on treatment systems implemented. A consolidated approach at international level and on which the system suggested in these guidelines is based, is the monitoring-actions sequence called ALF and hereunder described.

1.2.1. ALF systems

The ALF approach establishes a multistage-type model, organized through a series of measures envisaging several water controls through differentiated risk management measures and functional to the contamination risk level assessed on surface water (detection and alert levels) and to possible mitigation actions carried out within the water treatment chain. ALF general criteria are also used for reservoirs with an intended use other than human consumption, such as recreational or irrigation waters, according to a different risk assessment, that is functional to the specific water use. Consequently, in these kind of contexts, the decision basis, the safety limitations adopted and the actions taken, can also be significantly different from those presented in this document.

Taking into account the water for human consumption, it is useful to take a look to the approaches appeared in the last two decades and gradually move to the risk management model recommended at national level by the guidelines.

– *Burch system*
  The system suggested by Burch in 1993, uses, as elements for the implementation of differentiated measures, the number of cells detected in raw waters, and it defines three levels (11):
  - *Alert level 1:* reduced cell levels: 500-2,000 cells/mL;
  - *Alert level 2*: moderate cell levels: 2,000-15,000 cells/mL;
  - *Alert level 3*: high, persistent cell levels, more than 15,000 cells/mL.

  In levels 1 and 2, waters are considered as appropriate for human consumption while, in the absence of specific risk mitigation measures, level 3 states the non suitability for human consumption. Different actions are suggested based on alert stages, both from the surveillance (species-specific identification of algal population, cyanotoxins analysis) and solution (changes in the catchment depth, water treatments) point of view, together with recommendations on the decision-making process.

– *WHO system*
  Some years later, the WHO (7) reconsidered the ALF approach based on the assessment of cyanobacteria concentration detected in the source waters defining the three following stages:
  - *Surveillance level*: related to cyanobacteria detection which demands the enhancement of algal monitoring;

- *Alert level 1*: activated for cyanobacteria concentrations greater than 2,000 cells/mL (chlorophyll-a greater than 1 µg/mL), in which there is the possibility of cyanotoxins occurrence equivalent to the guide value (1.0 µg/L for MC-LR) and it is needed the activation of analytical measurement regarding cyanotoxins levels and the implementation of appropriate treatment measures for the removal of algal cells and toxins, followed by a report to the health authorities;
- *Alert level 2*: equivalent to concentrations greater than 100,000 cells/mL (chlorophyll-a greater than 50 µg/L) for toxic cyanobacteria, in correspondence of which, beyond monitoring enhancement and treatment systems enhancement/optimization, the emergency alternative water supply identification is carried out, followed by an adequate communication between health authorities and media.

– *CIMF system*
ALF principles have been integrated within more general management plans defined as Cyanobacterial Incident Management Framework (CIMF) (13) that envisage a more coordinated system based on regular monitoring, surveillance level and three levels of alert, where the passage from an alert stage to the next one is determined by the positivity of different indicators among which, beyond those aimed at the identification of algal cells and cyanotoxins, the bioassay is also suggested.

– *Australian system*
The Burch model (11) has been redefined and integrated in the Australian national protocol (14) for the monitoring of cyanobacteria and cyanotoxins in the surface waters establishing:
- *Detection level*: concentration of cyanobacteria greater than 500 cells/mL;
- *Alert level 1*: concentration of cyanobacteria greater than 2,000 cells/mL;
- *Alert level 2*: concentration of cyanobacteria greater than 5,000 cells/mL;
- *Alert level 3*: concentration of cyanobacteria greater than 50,000 cells/mL.

This system also uses the cyanobacteria biovolume measurement as an alternative to algal counts and it takes into consideration the identification of cyanotoxins in the last stages of alert as a criterion of risk assessment for water consumption.

– *Newcombe system*
A more recent evolution of the system based on WHO principles (12) and developed according to knowledge progress even in respect to toxins species-specific production potential, has been suggested by Newcombe (15) and it identifies a detection level and three different levels of alert. The definition of the different levels and the actions associated to each level of alert are hereunder briefly described:
- *Detection level*: concentrations of cyanobacteria roughly included in the range 500-2,000 cells/mL.
  This is useful to identify an early stage of algal bloom. Whereas in the water management system there is not an operative and appropriate monitoring of cyanobacteria, it is recommended to implement it on a weekly basis, thus integrating the information with frequent visual inspections of the water body so as to detect the presence of scum or water stains.
- *Alert level 1*: concentrations of cyanobacteria *Microcystis aeruginosa* in the range 2,000-6,500 cells/mL, in waters collected at the catchment.
  This is defined based on conservative criteria so as to ensure a time interval of 4-6 days, before the population development reach levels presenting cyanotoxins concentrations equivalent to the guide value (alert level 2). At alert level 1, it is

recommended to notify the situation to local health authorities and, if possible, to activate analytical determinations of cyanotoxins. Other decisions must be taken on a case by case basis according to the information available on species toxicity, the pre-existing scenario, with a particular attention to cyanobacteria proliferations episodes already occurred, the immediate availability of possible alternative supplies, the kind and effectiveness of water treatment plant.

- *Alert level 2*: it reports, in the absence of specific data on toxin levels, the possibility that the water entering the drinking water supply chain presents microcystins concentrations near to the guide value; the appraisal is conservative taking for granted that the algal population is highly toxic and that the entire toxin produced is released into the water and cannot be removed by treatments. The concentration of M. aeruginosa which defines level 2, is in fact calculated assuming, at worst, a part of toxin per cell (toxin quota) equal to 0.2 pg, that, taking into account a concentration equal to 6,500 cells/mL, would result in a toxin concentration equal to 1.3 µg/L, the guide value considered in the Australian guidelines (14). The appraisal clearly presents a high degree of approximation in that the toxin quota in natural populations of cyanobacteria is considerably variable and difficult to be defined and, furthermore, it is different from one species to another. Based on this, assuming in the appraisal the same criteria mentioned above and the toxin quota values referred to each species, in Australia there has been suggested a more specific evaluation for alert level 2 for the most spread algal species, according to the following values (15):
  - *Microcystis aeruginosa:* 6,500 cells/mL;
  - *Anabaena circinalis:* 20,000 cells/mL;
  - *Cylindrospermopsis raciborskii:* 15,000 cells/mL;
  - *Nodularia spumigena*: 40,000 cells/mL.

  This level of alert entails a decision on the notification to the health authorities and on possible use restrictions where there are no water treatment systems and it is not possible to regularly determine toxins concentrations. At operative level, a constant monitoring of cyanotoxins and cyanobacteria composition is suggested, at least on a weekly basis.
- *Alert level 3*: active for concentrations greater than 6,500 cells/mL; it is referred to *Microcystis aeruginosa* toxic cells and represents a potential toxin production in water for human consumption with concentration near or ten fold greater than the guide value.

  A notification must be sent to the health authority, if not previously sent, and an accurate risk assessment must be established, taking first of all into account the treatment measures taken and their suitability – both for technologies used, existing systems effectiveness and maintenance status – also considering the existence of sensitive users' categories. If risks mitigation measures cannot be considered as appropriate, use restrictions provisions and emergency response plans implementation are needed. In any case, a constant monitoring is demanded (frequency recommended 3-7 days), so as to highlight population decline and the reduction of toxin levels within a safety threshold. Specific measures, especially in case of water use restrictions, must be adopted so as to ensure an adequate communication with media and population, from the side of health authorities.

  The passage from a high level of alert to a lower one is determined by cyanobacteria population decline and/or by the adoption of risk prevention and/or mitigation measures that the health authority considers as appropriate.

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2. SURVEILLANCE, ALERT AND RISK MANAGEMENT NATIONAL SYSTEM FOR CYANOTOXINS MONITORING IN WATER FOR HUMAN CONSUMPTION

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According to guidelines general objectives, this section is aimed at providing the operative tools to support the risk management related to cyanobacteria presence, especially potentially toxic species, in water bodies used for drinking water production.

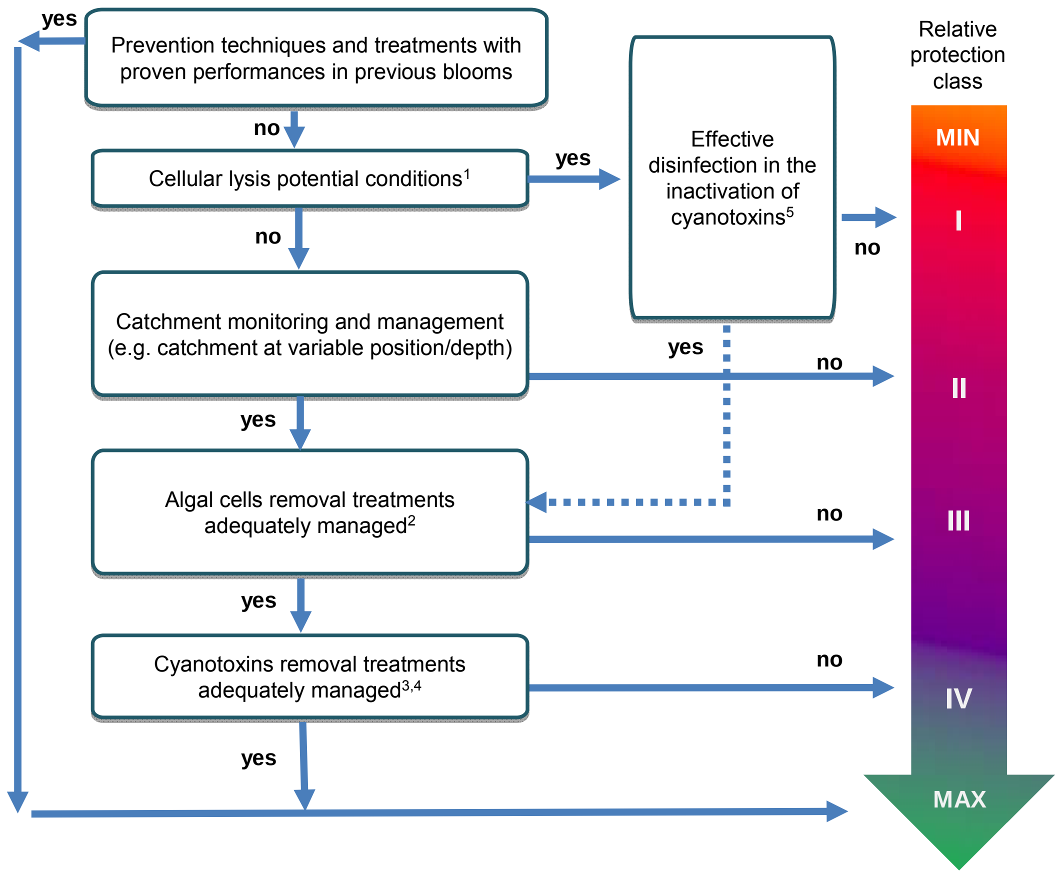
The criteria established by the working group and described in the present section, are mainly addressed to local health authorities, responsible for risk assessment and compliance determination on water for human consumption according to Legislative Decree 31/2001 and amendments and for water systems operators producing water for human consumption.

2.1. Surveillance and alert system

The suggested model is based on monitoring results of algal count carried out according to regulations requirements or planned by the operator and/or environmental or health authority, even according to the indications concerning potential system susceptibility and vulnerability to the presence and development of cyanobacteria populations. The monitoring should be intensified in reservoirs historically affected by blooms and it is crucial to take into consideration the seasonal blooming timing.

The established and suggested criteria – based on Alert Level Framework (ALF) principles developed and consolidated at international level – represent a decision tree, in which actions on water system monitoring and management are implemented in a progressive manner, as to give an answer to the algal development steps in water entering the treatment chain and taking into account risk prevention and mitigation measures related to the water supply system. Alert levels are defined through parameters related to the risk of cyanotoxins presence in the waters. In particular, for each level of algal concentration detected in the water body around the catchment point, a risk level can be established for the possible presence of cyanotoxins in drinking water. A series of modular measures based on this risk level exists and can include the enhancement of cyanobacteria and cyanotoxins monitoring frequency, the implementation and/or optimisation of water treatments, the notification to the health authority and, a last measure, the adoption of use restrictions of water resource and the activation of emergency plans.

This approach is based on the model represented in Figure 1, and on the summary scheme of risk levels referred to in Table A1 (attached to the chapter). The first one is applied to the drinking water treatment chain and describes the weight of the system protection level, in terms of prevention and mitigation of cyanotoxins presence risk in water delivery/user point; the second one is aimed at risk management in the entire catchment system until the distribution to water supply points.



[1] Some circumstances that can induce cellular lysis: algaecide treatments, pre-oxidation, algal population aging process, high pressure pumping (see Box 10-11)
[2] Some effective methods for the removal of cyanobacteria cells: coagulation and sedimentation or flotation, sand filtration, ultrafiltration (see Box 10-11)
[3] Some effective methods for the removal of cyanotoxins: filtration on activated carbon, ultrafiltration, ozone (see section 3.3 Box 10-11)
[4] The removal/inactivation system effectiveness depends on the technologies applied, plant size, water characteristics, entering algae and toxins levels, maintenance, etc. (see Box 10)
[5] The disinfection system effectiveness, depends from the technologies applied, plant size, water characteristics, entering algae and toxins, maintenance, etc. (see *Rapporti ISTISAN* 11/35 Pt.1 section 4.2.3.2)

Figure 1. Appraisal of water management system protection level

The criteria used for the definition of alert levels, risk level assessment, specific actions and protection and mitigation measures to be implemented, are described as follows:

– *Detection level 0*
  This level is aimed at highlighting, during a systematic monitoring, the possible occurrence of a bloom at a preliminary stage. The algal count levels, referred to toxic species, are approximately included in the range 500-2,500 cells/mL. At this stage, there is no immediate health risk evidence, even where organoleptic changes of the water are visible. It is recommended to intensify algal species monitoring within the water entering the plant, integrating the data with frequent reservoir inspections so as to detect possible

presence of scum or colour changes at different depths. To give an example, algal population development can reach concentrations associated to the alert level in a week or less (assuming a time period of almost 4 days for population doubling), based on environmental conditions.
As for the other system stages, it is assumed that the sample is collected near the catchment so as to directly refer to the risk for human consumption; other samples can anyway be collected from the reservoir and can be useful to study the possible distribution of cyanobacteria in the water column or in other water body areas or, in case of collection of scum with considerably high algal concentrations, to highlight the population toxicological profile in terms of composition and toxin levels produced, through confirmatory methods. For this purpose early-warning systems are very useful.

– *Detection level 1*
This level defines a cyanobacteria population and development within the water body able to reveal, in a highly precautionary scenario, a toxin production potential in water for human consumption with concentrations near to the guide value. The algal concentration values appraisal associated to the alert level, also defined as "risk surrogate" (1), are obtained through conservative criteria, considering the entire algal population as toxin producer, assessing a high value of toxin produced for each single cell and assuming that all the toxins produced are free in the water, represent the most toxic species and are not removed during treatments.
For the assessment, there have been used the criteria established by the World Health Organization (WHO) (2) and reconsidered by other authors (1, 3, 4), assuming a conservative appraisal of toxin value associated to each cell and establishing, from this last, the number of cells needed to obtain a concentration of toxins in the waters near to the reference value. As far as the *toxin quota* is concerned, reference should be made to section 2.2. and 2.4. of *Rapporti ISTISAN* 11/35 Pt. 1 for further in depth considerations and, in this context, an average value of 0.2 pg/cell (1, 3) is considered for the different toxic algal species. Therefore, in case of *P. rubescens* occurrence, based on the maximum precautionary principle, on the information spread in literature (1, 3, 5), which consider the category associated to a higher cyanotoxins production – indications confirmed by the various national monitoring data assessed by the working group – it is assumed the presence of a toxin quota equal to the double of the one established for the other toxic cyanobacteria species, taking as reference a cells threshold value consequently reduced.
The actions to be implemented in correspondence of alert level 1 include:
- notification to the local health authority, if the monitoring has been carried out by the operator (internal monitoring), by environmental agencies or by research groups;
- activation of a constant monitoring on a fortnightly or better weekly basis, depending on plant protection status and on resources availability, through algal count;
- risk assessment that can be associated to toxins potential presence in the waters going out from the drinking water treatment plant and for distribution, based on plant level protection system; in this case, if available, the historical data concerning the efficiency of plant during bloom, are crucial.

Protection assessment can be based, in general, on criteria reported in Figure 1. In this model, at alert level 1, a class I-II protection degree is considered to be inadequate, that can occur in small management systems. The assessment must anyway take into account the appropriate sizing of the plant and operation and maintenance general conditions (it can be useful to inspect plants, monitor the internal and external – ordinary and extraordinary – maintenance status, time intervals of plants backwashing, internal data related to the operative monitoring, compared, for example, to the operating pressures of filtration plants, etc.); at last, take into account the waters treatment obligation before their distribution for human consumption, in compliance with Legislative Decree 152/2006, concerning pre-treatments based on water body classification.

Whereas the plant protection level is considered inappropriate in any case, the first thing to do should be to implement cyanotoxins determination on a fortnightly or weekly basis; cyanotoxins determinations must refer to the overall content (intra- and extra- cellular) and they are generally performed on inlet and outlet waters and, where applicable, during distribution – in this case taking at least into consideration a proximal and distal point of the network where the water is not mixed; optimise, as far as possible, mitigation measures within the drinking water treatment chain thus ensuring an appropriate chlorination; this should be performed by increasing the contact time and, in the absence of other protection measures, by maintaining the residual chlorine levels in distribution at least at concentrations of 0.1-0.2 mg/L or even greater, if the health authority judges it to be necessary, at alert stage 1-2, taking also into consideration the monitoring results related to disinfection by-products.

At application purposes, based on accrued national experiences, it is crucial to consider that in a reservoir characterized by a fixed establishment of cyanobacteria, algal concentrations established for the level of alert are extensively exceeded during more or less prolonged time intervals within the year and, usually, they do not entail any use restrictions of water resource because adequately managed in the drinking water treatment chain. In fact, if the water system "coexists" with the more or less periodical occurrence of cyanobacteria, the above said measure, expressed in a general and conservative form, should be adapted to the context and optimised, also in terms of resources, based on the experience gained. In these cases, it is crucial that the water supply operator keeps the internal system documents highlighting the effectiveness of measures taken according to the historical data obtained in bloom conditions, including internal operative tests data such as for example the *jar test* for removing algal cells.

The monitoring frequency must be maintained until 2 consecutive results state a reduced risk level, after that the frequency can be gradually decreased.

– *High alert level 2*

Based on the values determined for the alert level, a *high alert level 2* is established to represent the development of cyanobacteria population in the water body able to reveal a potential toxin production in water for human consumption, with concentrations almost ten fold greater than the guide value. In this case, for risk management it is crucial to envisage adequate treatment measures within the water system so as to mitigate the risks; in the absence of these measures use restrictions and emergency response plans are needed. So as to integrate the measures described above for lower risk levels, at this stage it is recommended to:

- inform the local health authority about the phenomenon development;
- implement a constant monitoring on a biweekly basis through algal count;
- determine, on a weekly or (better) biweekly basis, the presence of cyanotoxins in waters entering and going out from the purifier and in distribution (at least a proximal and distal point of the network where the water is not mixed);
- optimize mitigation measures in the drinking water treatment chain, thus ensuring an adequate chlorination;
- be prepared for a possible emergency response plans implementation (see section 3).

As far as application purposes are concerned, based on accrued national experiences, it is crucial to consider that in a reservoir characterized by a fixed establishment of cyanobacteria, algal concentrations established for the high level of alert can be overcome in mainly short periods during the year and, if correctly managed, can avoid use restrictions of water resource. Even in these circumstances, if the water system is repeatedly affected by cyanobacteria, the above said measures must be adapted to the context and optimized, even in terms of resources, based on the experience gained.
The described plan, necessarily offers a scheme of three representative risk levels. Intermediate situations (e.g. for algal concentrations between 5,000 and 50,000 cells/L) will be managed through appropriate decisions proportioned to risk level, to be assessed on a case by case basis according to the general scheme.
The monitoring frequency must be maintained until two consecutive results state a reduced risk level, after that the frequency can be gradually decreased.

2.2. Measures and use restrictions

The current state of knowledge on risk assessment related to the presence of cyanobacteria massive expansion in water for human consumption, as described in the previous sections of these guidelines, points out that the health risk is only associated to the production of water for human consumption, contaminated with cyanotoxins levels exceeding the guide values. It is also useful to remember that the reference values definition concerns a chronic exposure, that is a prolonged consumption, officially, "during the entire life cycle", of water contaminated with cyanotoxins levels exceeding the reference values.

Based on this, the working group recommends use restrictions of drinking water after having observed a concentration of toxins exceeding the maximum acceptable values in water for distribution.

The maximum temporary acceptable value for the MC-LR in water for human consumption is equal to 1.0 μg/L referred to the total toxin content (intra- and extra- cellular). According to an extensively conservative approach for human health protection, with an overestimation in the toxicity assessment, in the worst appraisal approach, the 1.0 μg/L value must be referred to the sum of different MCs congeners concentrations in the sample, considered as equivalent of the MC-LR. For this purpose, there have to be found microcystin congeners elements through confirmation methods using the best analytical potential available and, as minimum criterion, the congeners elements for which analytical standards are now commercially available, [D-Asp^3]-MC-RR, MC-RR, MC-YR, [D-Asp^3]-MC-LR, MC-LR, MC-LA, MC-LY, MC-LF, MC-LW. Cyanotoxins research must be extended to other compounds categories, such as for example Cylindrospermopsin and anatoxin, in the presence of blooming (for an alert greater than 1) of species producing these toxins; the maximum acceptable value of cyanotoxins other than microcystins can be fixed in compliance with the provisions of Legislative Decree 31/2001, art. 11(1)(b).

In case of a contamination exceeding the maximum acceptable value, the use restrictions concern, in general, just the drinking water use and the preparation of foods where the water is the main ingredient, with particular respect to risk categories. In case of use restrictions related to the exceeding of reference limits there is the need to activate emergency response plans, to implement alternative water supply and to arrange an epidemiological monitoring centre, communication methods and anything else described in section 4-6 of this volume.

However, as far as the adoption of use restrictions is concerned, art. 10(1) of the Legislative Decree 31/2001 has to be considered for the need to take into account the exceeding level and potential risks for human health as well as the risks that could derive from an interruption in the supply or from a use restriction of the delivered water; the possible use restrictions must first of all envisage the recourse to alternative supplies and the urgent implementation of adequate treatments to restore the compliance of the distributed waters.

2.3. Specific measures recommended based on ALF system

In the previous section there have been presented the criteria and strategies according to which it is possible to assess the cyanotoxins risk degree in the production and distribution chain of water for human consumption and establish possible solutions aimed at avoiding consumers exposure.

The elements at the base of system development and implementation, in particular as far as the risk assessment is concerned, have been deepened in the volume "Cianobatteri in acque destinate a consumo umano. Stato delle conoscenze per la valutazione del rischio".*

It is nevertheless useful, in this section, to recall the key elements and the actions recommended for the different strategic aspects driving the ALF implementation. For this purpose, Table 1 indicates the main aspects of system structuring and implementation concerning, in particular, risk assessment for the specific water system, surveillance and monitoring methods implemented, emergency response and communication measures. For each determinant considered, a reference exists to a specific in depth box. In every box presented (in Appendix A), for each of the elements determining the ALF system there is:

– a short summary concerning the state of knowledge;
– some specific recommendations within risk prevention and management context;
– the corresponding in depth section, concerning the elements involving risk assessment and surveillance and monitoring methods or, in this same volume, the aspects related to the emergency solutions.

Table 1. Key aspects of ALF system

ALF system determinant		Box*
Likelihood of occurrence of toxic phenomena in the reservoir and in the water system and risk assessment	Environmental factors lying under cyanobacteria proliferation	1
	Toxic species and impact on water for human consumption in Italy	2
	Toxins associated to cyanobacteria	3
	Regulation and reference values	4
	Water system analysis	5
Surveillance and monitoring methods	Toxic cyanobacteria identification	6
	Early warning controls at catchment	7
	Cyanotoxins identification methods	8
Risk mitigation measures	Prevention measures: controls at catchment and on nutrients	9
	Removal of cyanobacteria and their metabolytes	10
	Cyanotoxins degradation products	11
Emergency response	Emergency response plans	12
	Epidemiological surveillance system	13
	Risk information and communication	14

* Boxes are represented in Appendix A of this volume

* *Rapporti ISTISAN* 11/35 Pt 1.

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Annex 2.1.

Table A1. Summary scheme concerning risk levels adopted in the surveillance system

Decision basis	Threshold definition and expected risk[1]	Recommended actions	Measures and possible use restrictions[2]
0 Detection level			
Detection of potentially toxic cyanobacteria presence during monitoring[3,4]	Potentially toxic species [4,6]: 500-2,500 cell/mL *or* Cyanobacteria chlorophyll: 1-2.5 µg/L *Cyanobacteria detected at low level. No immediate health risks.*	To intensify reservoir visual inspection. To implement a regular monitoring, at least on a fortnightly basis, of algal or cyanobacteria chlorophyll count[6,11,12]	-
1 Alert level			
Alert for possible health risk: algal count associated to a potential presence of cyanotoxins in waters for human consumption (before drinking water treatment chain) to a level equal to the maximum acceptable value[7,8]	*P. rubescens:* 2,500 cell/mL[4,6] *or* Other toxic species: 5,000 cell/mL[4,6] *Settlement and development of cyanobacteria population in the water body so as to envisage, in the worst possible scenario[7], a potential production of toxin in waters for human consumption with concentrations near to the maximum acceptable value (1.0 µg/L MC-LR[8])*	Notification to local health authority in case of recurring/systematic and adequately managed phenomena[5]. To implement a continuous monitoring on a biweekly basis or better weekly basis, through algal count [6,11,12] at least on system inlet and outlet waters. If system protection level is considered as inadequate[9], there is the need to implement the analysis, on a weekly basis[9,10,11,12], of cyanotoxins in inlet waters system, and if necessary, in outlet waters and/or in distribution[13]. To optimize, as fare as possible, mitigation measures in the drinking water treatment chain[9,14] To ensure an adequate chlorination[15]	Use restrictions[2] following the detection of toxins concentrations exceeding the maximum acceptable values in distributed waters[8,13]
2 High alert level			
High alert level for a possible health risk: algal count associated to a potential presence of cyanotoxins in waters for human consumption (before drinking water treatment chain) to a level equal to 10 fold the maximum acceptable[7,8] value in waters for human consumption	*P. rubescens*: 25,000 cell/mL[4,6] *or* Other toxic species: 50,000 cell/mL[4,6] *Settlement and development of cyanobacteria population in the water body so as to envisage, in the worst possible scenario[7], a potential production of toxin in waters for human consumption with concentrations near to 10 fold the maximum reference value (1.0 µg/L MC-LR[8]). Adequate prevention and treatment measures must be implemented so as to mitigate risks, otherwise use restrictions and emergency response plans[16] together with an adequate training and communication[17] are demanded*	Notification to local health authority[5] Continuous monitoring on a weekly basis, or better biweekly basis, through algal count[6,11,12] Cyanotoxins identification on a weekly or better biweekly basis[11] on inlet and outlet waters from the water treatment system and in distribution[8,10,11,12,13] To optimize and/or enhance mitigation measures in the drinking water treatment chain[9,14] To ensure an adequate chlorination[15] Organization of emergency[16], information and communication[17] plans	Use restrictions[2] following the detection of toxins concentrations exceeding the maximum acceptable values in distributed waters[8,13]

1. Threshold is the maximum acceptable level of toxins concentration in the reservoir water entering the water management system (as preventive measure it is equal to the maximum acceptable value of cyanotoxin in the water at consumer site), assessed from cyanobacteria level in waters, according to a conservative assessment of potential cellular production level (toxin quota); the expected risk is considered as the potential occurrence in the water distributed for human consumption of cyanotoxins contamination level near or exceeding the maximum acceptable value.
2. In use restrictions adoptions must be however taken into consideration art. 10(1) of Legislative Decree 31/2001 and amendments, concerning the need of considering the excess amount and potential risks for human health, as well as those risks that could derive from a supply interruption or from a use restriction of distributed waters; the possible use restrictions must first of all envisage the recourse to alternative supply and the urgent implementation of adequate treatments. Other details in section 2.1.1. *Rapporti ISTISAN* 11/35 Pt. 1 and Box 4 and 14 of this volume.
3. The routine monitoring on the reservoir must be envisaged according to the regulation in force (see section 2.1. *Rapporti ISTISAN* 11/35 Pt. 1, Box 4 of this volume) or to risk plans if envisaged by the water operator based on a risk assessment according to the WSP approach.
4. Values established on samples collected near the catchmet or in inlet waters (raw waters), where the sampling in the reservoir is not possible to carry out; other assessments can be performed according to the algal count of samples collected in other areas and/or water body deepness, and which highlight a possible concentration equal to the threshold values established for the area near the catchment (taking for example into consideration the likelihood of vertical shifting on the water column of algal mass); in-depth analysis in section 2.1 and Appendix B in *Rapporti ISTISAN* 11/35 Pt. 1, and Box 1-2, 6-7 of this volume.
5. If the risk related to cyanobacteria occurs in the water system on a regular or systematic and/or prolonged basis (in general occurring in two or more months during which the alert level 1-2 persists), data of internal monitoring concerning the risk due to cyanobacteria and toxins, including monitoring data, can be provided by the operator through methods and timings to be agreed with the health authority on a case by case basis, according to contamination level, prevention and mitigation measures adopted by the plant (according to plant size), technologies used for water treatment, level of systems maintenance and monitoring) and to the experience gained by the water system; as a general rule, for water systems with multiannual experience in risk management using adequate and tested monitoring systems, a communication of data on a six-month or annual basis can be envisaged (summary of cyanobacteria concentration data and, if necessary, of toxins levels in inlet, outlet and distribution waters) together with the realized interventions, except for extraordinary contamination events.
6. For further details on potentially toxic species please see section 1.2. and 1.4. *Rapporti ISTISAN* 11/35 Pt. 1 and Box 2 of this volume.
7. Maximum precautionary criteria by considering the entire algal population as a toxins producer, assessing a high level of toxin produced for each single cell and assuming that all the toxins produced are free in water, represent the most toxic varieties and are not removed during treatments.
8. The maximum acceptable value for cyanotoxins, in the point of compliance established by the Italian Legislative Decree 31/2001 (art. 6), is actually fixed for MC-LR at 1.0 µg/L, as sum of different microcystins congeners concentrations, expressed as MC-LR equivalent. The microcystins congeners identifiable at the best of analytical potentiality available, must be found through confirmation methods, and as minimum criterion congeners elements for which analytical standards are commercially available at the current state: [D-Asp^3]-MC-RR, MC-RR, MC-YR, [D-Asp^3]-MC-LR, MC-LR, MC-LA, MC-LY, MC-LF, MC-LW. Cyanotoxins research must be extended to other compounds, such as for example, cylindrospermopsin and anatoxin in bloom presence (alert greater than 1) of potential producers of these toxins; the maximum acceptable value of cyanotoxins other than microcystins should be fixed in compliance with Legislative Decree 31/2001, art. 11, clause 1, b; in-depth analysis in section 2.3. *Rapporti ISTISAN* 11/35 Pt. 1 and Box 4 of this volume.
9. A class I-II protection level, as indicated in Figure 1, can be considered as inadequate; yet the adequacy of system size, functioning and maintenance conditions should also be assessed; moreover waters treatment is also required before they can be delivered for human consumption as stated in the Legislative Decree 152/2006 concerning pre-treatments based on water body classification; further details in section 4. *Rapporti ISTISAN* 11/35 Pt. 1, Box 5, 9-12 of this volume.
10. Cyanotoxins determinations must be referred to the overall content (intra- and extra-cellular); the analytical method must be adequate for the purpose (see section 3.3, Appendix A in *Rapporti ISTISAN* 11/35 Pt. 1 and Box 8 of this volume).
11. Monitoring frequency must be established according to the assessed risk level (first of all algal concentration and toxin levels in inlet waters and where necessary in outlet and distributed waters, assessment of the reduction through the system during the monitoring and other operative indicators such as for example turbidity, TOC, etc.).
12. To keep monitoring frequency until two consecutive results state a reduced risk level, after that the frequency can be gradually decreased.
13. As far as distributed waters monitoring is concerned, it is required to take into consideration a proximal and a distal point of the network where the water is not mixed with water having other origins.
14. Protection measures III-VI (Figure 1) must be implemented for concentrations exceeding alert level 1; further details in section 4. *Rapporti ISTISAN* 11/35 Pt. 1, Box 5, 9-12 of this volume.
15. About 0.1-0.2 mg/L of residual chlorine, increasing, where possible, the contact time during disinfection; higher chlorine concentrations (e.g. 0.5 mg/L) can be recommended for short periods of time during an emergency, taking however into consideration that the monitoring of disinfection by-products can be critical due to the increase of organic substance charge caused by algal mass in inlet waters; it is therefore required to monitor the levels of disinfection by-products such as trihalomethanes and/or chlorite according to the techniques adopted for the possible pre-oxidation and post-disinfection; in-depth analysis in section 4.2. and 4.3. *Rapporti ISTISAN* 11/35 Pt. 1, Box 10-11 of this volume.
16. See section 3-4 of this volume.
17. See section 5 of this volume.

3. EMERGENCY RESPONSE PLANS

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The state of emergency is represented through the evidence of a health risk for the consumer due to the presence in the water of cyanotoxins levels exceeding the maximum acceptable values. It has to be highlighted that, in many cases, the occurrence of a state of emergency is very rapid, even few days, and the time frame in which the emergency occurs usually involves a few weeks.

It is therefore clear that a safety management of the emergency, the health, economical and social impact of these phenomena over consumers, is related to interventions promptness and actions suitability. Those aspects therefore need:

- a previous preparation of the emergency plan;
- a coordinated and planned engagement of all the parties involved during the emergency stage as far as suitable procedures are concerned;
- preparation of human resources, through an adequate training.

It has to be noticed that emergency plans have already been established by every operator of water systems for human consumption, and different scenarios having effect on the water utility, such as for example, extreme climatic events or potential hostile actions against the system, have already been considered. Plans define, in general, each duty and responsibility and envisage the creation of a "crisis unit" within companies and identify emergency equipments, such as power units, movable tanks and tankers, water provisions and baggers, means of transport, operative room equipment, etc., also through coordination among Companies of the same Province/Region, possibly coordinated by Prefectures.

The emergency response plans described in the present section can usefully include crisis management measures already established by water supply systems.

3.1. Technical roundtable

The preventive organisation of a technical roundtable may grant, during an emergency stage, the presence of a multidisciplinary and coordinated expert team able to manage the different crisis stages at its best.

Ideally, the technical roundtable team must ensure:

- the involvement of local key functions, at health and environmental level, water supply operators, basin authority and other interested parties;
- the presence of experts of different disciplines such as biology, chemistry, toxicology, medicine, hydraulic engineering, agricultural science;
- a support, if needed, by the side of national institutions, such as for example members of the cyanobacteria national group, so as to quickly provide crucial information, scientific and technical tools;
- the availability of live rich data on which the decision-making process must be based;
- information, communication and transparency of decisions.

Table 1 shows, as example, the armed task force created to deal with a drinking water emergency due to cyanobacteria which involved, in 2009, the reservoir of Occhito (1) used for several purposes among which the catchment of water for human consumption in a large area of the Apulia region.

Table 1. Task force created to deal with the drinking water emergency due to cyanobacteria which involved, in 2009, the reservoir of Occhito

Body	Functions, role, contribution
Regional Authority	Coordination
Province	Information on sensitive consumers, risk perception, economic activities and other specific issues
Mayors of municipalities involved	Information on sensitive consumers, risk perception, economic activities and other specific issues Beneficiaries of local measures
Civil Defence	Definition of possible water supply measures in state of emergency
Local health authority	Responsible, at institutional level, for the decisions on risk assessment and suitability of water for human consumption
Istituto Superiore di Sanità	Technical and scientific information support, opinion on solutions suggested Determination of toxins levels in waters
Regional Authority Environment Protection	Data on the qualitative-quantitative composition of algal species in the basin, in raw waters, in inlet and outlet waters from the purifier, and in distribution
Development Consortium Basin Authority	Data on the hydrodynamic regime of the basin and water use Implementation of established basin management measures (e.g. basin levels, sediments analysis)
Water supply operator	Data on internal controls Prevention and mitigation measures for the risk in drinking water supply chain implementing body
Epidemiological monitoring centre and first aid service	Arrangement of observations over possible syndromic frameworks due to consumptions of contaminated water
Regional communication office	Informing the population

3.2. Decision-making information

The availability of consolidated information during the emergency stage, is useful to grant the correct response time for definite needs and decisions. For this purpose, the following information is basically needed:

– list of key roles (organisations, institutions, people) and main contacts (e.g. active carbons suppliers);
– main procedures for events management;
– main logistic and technical information, such as the localization of possible alternative water supplies and related rate.

3.3. Decision-making process

According to the experiences gained, it is deemed to be useful to recall, as an example, the decision-making process occurring during the emergency stage and that has to be supported through adequate technical-scientific knowledge:

- situation analysis and hazards objective assessment;
- identification and involvement of key functions in monitoring actions, control over decisions concerning supply methods;
- identification of actions priorities such as the integration of water treatment systems with other practices and technologies;
- definition/selection of possible options and impact analysis in terms of cost-benefit;
- establishment of additional water supply measures;
- alert measures at Civil Defence for water supply during the emergency stage;
- risk mitigation adequate treatments (e.g. integration with GAC of pre-existing sand filters);
- communication to consumers and users;
- planning of mid-long term actions such as the analysis of factors favouring the algal bloom;
- assessment of risks related to use other than human consumption such as agriculture, fishery, recreational uses, food production.

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4. EPIDEMIOLOGICAL MONITORING CENTRE AND SYNDROMIC SURVEILLANCE MODELS: SYSTEM STRUCTURING AND FUNCTIONING

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4.1. General aspects

Cyanobacteria presence in water supply sources is reported in several Italian regions since the 1980s (1). Already in 1989, on the Lake Garda surface, large *Anabaena lemmermannii* and *Planktothrix rubescens* summer blooming have been registered (2). Later on, at the end of summer 1997, in the water of the lake Iseo, a significant bloom of *Anabaena flos-aquae* was documented (3).

In the following years, cyanobacteria blooms have been observed in the lakes of Marche (4), Sardinia (5), Umbria, Latium (4) and Emilia Romagna (6) regions.

In the last decade the number of evidence related to the presence in internal waters, especially lakes, of cyanobacteria blooms associated to surface water bodies eutrophication conditions due to nutrients introduction, especially phosphorus or nitrogen, were intensified.

Starting from winter 2008-2009, also the Occhito Lake, at the borders between the Province of Foggia and Campobasso, was affected by a large algal bloom, concerning the *Planktothrix rubescens* species (7, 8), with the consequent contamination of water for irrigation use, human consumption and mussel farming. This event, occurred in a context traditionally poor of water resources as the Apulia region, has determined a real crisis related to the possible restrictions of drinking water supply to large territories of the Province of Foggia.

The effects on human health related to cyanotoxins exposure have been extensively documented and the spreading of cyanobacteria bloom within water bodies need the creation of an epidemiological surveillance system over exposed populations. Healthcare events reporting, especially when compatible with a formal definition of event, by the side of healthcare operators or laboratories, allows public health services to implement all the measures needed to avoid a further disease spreading.

This surveillance system must take into account the following elements:

- extent of environmental risk;
- possible exposure modes;
- different pathologies related to exposure.

4.2. Environmental risk

The environmental surveillance will be based on a monitoring system regarding the presence of cyanobacteria in water basins of relevant territories. For the environmental monitoring the WHO (World Health Organization) recommends the ALF (*Alert Level Framework*) model against cyanobacteria blooms, which was developed for the first time in Australia in 1991 (9).

This system is based on the identification of 4 levels (detection level, alert level 1, 2 and 3), scanned according to cyanobacteria cells concentration, that is, to the presence of *Microcystis aeruginosa* or total biovolume of the overall number of cyanobacteria. The national surveillance system described in section 2, is based on this.

Depending on surveillance objectives and on the possible need to implement prophylaxis measures, it will be necessary to arrange systematic and historical series of data or updated and prompt data.

4.3. Exposure modes

The main exposure modality to cyanotoxins occurs orally, through voluntary swallowing of drinking water or foods (fishery or agricultural products) or accidentally/involuntarily swallowing of recreational water. The exposure can also occur through skin or inhalation.

4.4. Pathologies related to exposure

The effects on human health related to cyanotoxins exposure can be based on exposure and latency time, acute or chronic.

The LPS endotoxins (Lipopolysaccharides) on cyanobacteria cell-wall, can determine skin and mucosal irritation, as reported in a trial carried out on volunteers exposed to *Microcystis aeruginosa*, *Aphanocapsa incerta* and *Cylindrospermopsis raciborskii* (10) cultures. Moreover, LPS endotoxins can be pyrogenic (11) thus determining gastrointestinal symptoms (12).

Cyanotoxins associated to cyclic peptides group (microcystins and nodularin), are known for causing hepatic damage through the inhibition of phosphatases (13). In particular, very high amounts of these toxins can lead to death due to acute liver failure in a few hours or some days, as happened during a microcystin intoxication epidemic in dialysis water within a group of patients treated with haemodialysis in Brazil in 1996 (14). The chronic exposure to these toxins could be associated to the hepatocellular carcinoma occurrence (13).

Cyanotoxins associated to the alkaloids group include both neurotoxins, such as anatoxin and saxitoxin, and cylindrospermopsin, of which a hepatotoxic action and a generally cytotoxic action have been documented. All cyanotoxins included in this group, can be deadly in high amounts because they inhibit breathing; in particular, anatoxins cause respiratory cramps and saxitoxin induces paralysis. However, there is no evidence of deaths related to alkaloid cyanotoxins exposure. Both the aplasia toxin, produced by *Lyngbya*, *Schizothrix* and *Planktothrix*, and lyngbya toxin, produced by *Lyngbya,* can have an acute irritation effect at skin level. The exposure to cylindrospermopsin could be associated to a renal failure (15, 16). A chronic effect of tumour progression has been attributed to lyngbya toxin and aplasia toxin, while cylindrospermopsin seems to play a possible genotoxic and carcinogenic role (17).

In the end, the exposure to β-N-methylamino-L-alanine (BMAA), produced by different cyanobacteria species, seems to be associated to the development of neurodegenerative disease such as amyotrophic lateral sclerosis (18-21).

4.5. Interinstitutional cooperation for surveillance activities

For integrating, within the epidemiological surveillance system, epidemiological and environmental data, it will be necessary to establish appropriate synergies among the institutions responsible for environment protection (Environment and Territory Protection Agencies), the structures in charge of epidemiological surveillance (Epidemiological Monitoring Centres, Epidemiology Units within Local Health Service Agencies) and the Local Health Authority (Mayor supported by the Hygiene and Public Health Service) which has to implement possible restrictions and limitations to protect public health. This agreement can be made official through the creation of inter-company or regional work groups or approvals and permits procedures, as established in section 3.

Just to give an example, reference can be made to what happened in the Apulia region during the Occhito Lake water emergency, where through Resolution no 48 as of 03.09.2009, the Planning of Territorial Assistance and Prevention Service Manager has established an Interinstitutional Technical roundtable for monitoring the *Planktothrix rubescens* algae phenomenon in the reservoir of Occhito and in the distribution network of the Province of Foggia, composed by the Health Policies Department Service managers, representing the Acquedotto Pugliese, the Civil Defence, the Territorial Government Office, the Province, the Basin Authority, the Consorzio di Bonifica della Capitanata, the Regional Agency for Environment Protection and relevant Local Health Authority (22).

4.6. Epidemiological surveillance of cyanotoxins chronic exposure effects

Where the waters affected by cyanobacteria blooms in surface water bodies are used for drinking purposes, there can be a chronic exposure risk. However, this event is to be considered unlikely, since the treatments to which surface waters are subjected before their distribution are carried out so as to obtain a strong reduction of cyanobacteria cells levels and dissolved cyanotoxins (23). Moreover, to this day, there are no *ad hoc* studies on chronic toxicity due to cyanotoxins; in literature there are just some ecological studies on cyanotoxins exposure chronic effect.

A study carried out by Svircev *et al*. examined the incidence and mortality related to hepatocellular carcinoma in certain Serbia regions, among which one was affected by episodes of cyanobacteria bloom in water basins with documented presence of microcystin. This study pointed out that both mortality and incidence of hepatocellular carcinoma were higher in this last region, thus assuming a possible correlation between the pathology and cyanobacteria presence in the basins (24).

A study carried out by Pilotto *et al.,* held in Australia, was aimed at assessing the relation between exposure to contaminated waters and cyanotoxins in the first pregnancy quarter and some outcome such as low birth weight, gestational age and prematurity, deduced from series of birth historical data. The study revealed how the greater exposure to cyanotoxins in the first pregnancy quarter may determine a higher incidence of low birth weight newborns, premature and babies with birth defects. However, no linear relation between dose and response has been shown by this study (25).

Caller *et al.,* through the exam of routine health flows, proved that in inhabitants living near the Lake Mascoma in Enfield (USA), which was contaminated with cyanobacteria producing BMAA, the incidence of amyotrophic lateral sclerosis was 10 to 25 fold higher than expected based on the national average (21).

Therefore, at present, the most useful study design for the assessment of cyanotoxins chronic exposure is the ecological one, due to the difficulty to correctly assess the individual exposure to this kind of toxins for a prolonged period of time and, on the other hand, the simplicity in the carrying out of those studies.

Even in Italy this survey method could be repeatable through the use of flows as cancer registry, CeDAP (*Certificati di Assistenza al Parto* - Delivery Assistance Certificates), nominative registers of death causes and hospital discharge form records, and the availability at Regional Agencies for Environment Protection, of historical series of results deriving from exams performed on water basins for cyanobacteria and cyanotoxins detection.

However it is necessary to remember that, traditionally, the ecological studies role has been the one of explorative analysis, where it is assumed that considered risk factor exposure levels are consistent within the entire population, circumstance that is not necessarily verifiable in case of cyanotoxins exposure. Moreover, these studies are particularly influenced by the confusion associated to the correlation among different environmental exposures; for example, in water where there is the presence of cyanobacteria, the existence of other microbiological and chemical risk factors that could be related to the health outcome searched, could be favoured (26).

4.7. Epidemiological surveillance of cyanotoxins acute exposure effects

At this date, some studies on acute toxicity on mouse are available, as far as cyanotoxins exposure is concerned; in particular the target organ most sensitive to the exposure seems to be the liver (27, 28).

It is possible to find cyanotoxins concentrations in drinking water causing acute effects due to cyanobacteria blooms or scum, malfunction to drinking water treatment system, use of cleaning agents releasing high quantities of intracellular cyanotoxins not subsequently detected, or supply through raw water non subject to drinking water treatments.

Literature contributions on monitoring of cyanotoxins acute exposure outcomes are scarce or anecdotic as well. From 1949, some case-report described a series of conditions associated to the exposure to cyanobacteria in bathing water: temperature, rash, gastrointestinal symptoms, headache, myalgia, pneumonia, dizziness and stomatitis. In 2006, Stewart *et al.* carried out a prospective cohort study so as to assess the incidence of acute symptoms related to individual exposure to cyanotoxins in the water of Queensland and New South Wales (Australia) and central Florida lakes (29). Study participants have been recruited during a three-year period (1999-2002) among those who attended lakes for recreational reasons; in particular, the recruitment has been carried out in 54 days, mainly during the summer season. Participants have filled in a background questionnaire and then have been called by phone. In the preliminary background questionnaire, demographic variables, chronic disease and recent acute disease were examined. At the time of follow-up the occurrence of respiratory, skin, ocular and gastrointestinal symptoms have been examined. On the same days, the analyses for the detection of cyanobacteria and cyanotoxins presence in lakes attended by the interviewed bathers were carried out. The survey highlighted that the respiratory symptoms frequency was higher in those subjects exposed to waters with the highest level of cyanobacteria contamination.

In Australia, in 2007, a survey carried out through questionnaires sent via postal service and involving 5,000 residents in the Deception Bay/Bribie Island area and in Northern Moreton Bay (Queensland), area affected by the *Lyngbya majuscola* bloom, pointed out that 34% of subjects exposed to marine recreational activities reported at least a respiratory, skin or ocular symptom, with skin symptoms at the highest level (30).

Osborne and Shaw, through the analysis of first aid access in Fraser Island, showed that during *Lyngbya majuscola* bloom, an access excess has been observed especially for skin symptoms (31).

In light of the international experiences reported, the monitoring of cyanotoxins exposure acute effects can be performed through prospective or retrospective cohort studies which have the undeniable advantage of methodological accuracy but are relatively useful for public health inasmuch they do not allow to gain timely information supporting health authority decisions.

Likewise, it is not possible to use, in case of cyanotoxins intoxication, a surveillance system based on notification systems of infectious disease relying on the indication of cases with diagnoses defined at clinical or laboratory level. In fact, in the event of cyanotoxins intoxications, the clinical diagnosis shows uncertainty margins or, it is carried out too late compared with the occurrence of symptoms that are common to other pathologies.

For this reason, in several contexts there have been already established surveillance systems able to register those cases of subjects affected by several aspecific signs and symptoms that represent not a specific pathology but a syndrome.

The aim of those systems is to prematurely identify potential threats for public health, so as to implement a rapid response (32).

During the algal bloom in the Occhito Lake occurred between 2008 and 2009, a syndromic surveillance system, based on the constant monitoring of first aid services access, continuing care and general medicine, has been experimented. This system relied on the notification to the Hygiene and Public Health Service of cases with suspected cyanotoxins intoxication, through the dedicated web portal or paper information flow.

Within this system the following have been defined:

– surveillance reference territory, identified in those Municipalities including in their area lakes affected by episodes of cyanobacteria bloom or supplied with water resources potentially polluted;
– time set in which the surveillance must be carried out, established taking into account the environmental monitoring data and the factors influencing the seasonality;
– case definition developed according to the knowledge of toxicity mechanisms of cyanotoxins present in the relevant water resources.
– a simple data transmission method (web-based);
– method of information return to all the operators involved in the surveillance.

The detection of events frequency higher than the expected threshold, established according to the available historical data, activated the health alert.

For example, if one takes into account that cyanobacteria bloom is more frequent during the summer, when temperature episodes accompanied by myalgia is basically low, a sudden increase in weekly first aid access or general medical examinations due to feverish syndromes with myalgia in a population potentially exposed to cyanotoxins, could entail an increase in the concentration of these last in the water basins.

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5. RISK INFORMATION AND COMMUNICATION

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Communication represents a crucial aspect within the wider risk management process and it involves each stage of this complex conceptual scheme: from the identification of hazard situations, risk assessment and exposure evaluation, to the establishment of surveillance and prevention intervention priorities, to the implementation of risk mitigation and emergency response measures (1).

The World Health Organization European Regional Office (WHO/Europe) recommends to separate every single step, so as to maintain process transparency and to activate an effective communication among all the participants in each stage of the same process (2).

It is therefore appropriate that reference Institutions choose to communicate among each other since the beginning and during all steps of the decision-making flow, according to strategically established methods, avoiding communicating only in case of emergency thus implementing wrong interventions and losing credibility.

The planning of communication initiatives represents a crucial requirement for granting focused interventions, agreed among subjects and Institutions involved, monitored and aimed at the achievement of communication objectives clearly identified in the plan strategy.

The carrying out of a communication plan represents an important institutional recognition of the communication role seen as a strategic, voluntary and relevant intervention. Moreover, planning underlines the Institutions engagement in promoting exchange and interaction among the different figures involved in the risk situation, both as active subjects in risk assessment and management and as exposed citizens, thus favouring the participation in decision-making processes and the sharing of choices.

Today, even in the health risk management associated to environmental issues, it is preferred a participatory communicative approach, based on information, perceptions and choice sharing among the different partners and on “autonomy strengthening” (3).

Therefore, risk communication strategies based on information flows from up to down (4, 5), characterized by unidirectional passage of knowledge and decisions that is believed can be accepted independently from its own will, are basically avoided; these strategies are accompanied and/or replaced by strategies relying on dialog and active and integrated participation.

On the other hand, only through a communicative exchange it is possible to foster this complex interactive process among central scientific Institutions, local community, mass media and citizens, thus favouring not only information and risk scientific assessments flow but also opinions, individual and collective perceptions circulation of worries and reactions.

The talks among parties and the participation represent a crucial condition for the awakening of citizens awareness on the relevant environmental issue and for the overcoming of a double risk: scaremongering and fatalism (6).

According to this, it should be, therefore, better to talk of communication “on” risk more than “risk” communication, inasmuch the risk does not only represent a message content that an expert subject send to non-expert subjects, but a topic around which, in a perspective of a social construction of the same risk, the parties involved (stakeholders) communicate with each other and establish agreed jointly strategies so as to face and manage the risk (7, 8).

Therefore, even the application of the Precautionary Principle through the implementation of risk protection and mitigation measures by the public structure, not only has to be considered as a preventive intervention, but also as an initiative aimed at the development, within the community, of an active and informed participation in risk management process (9).

5.1. Information and communication concerning cyanobacteria risk management

The communication concerning health risks has been established as interactive exchange of information and opinions among individuals, groups and institutions, among subjects involved in health risk assessment and management. The stakeholders that can take part in the decision-making process and have different objectives play different roles and have non-homogeneous skills and perceptions (2, 10, 11).

This communicative approach, so important for the management of all phenomena representing a risk for human health and involving the relevant Institutions, public and private organizations, single individuals and the community, not only take into consideration the technical-scientific information on risk nature, but also emotional factors related to the reactions of different partners. In order to operatively implement this approach, it is necessary to envisage an agreed planning of the initiatives, clearly establishing objectives, target involved, tools, time and criteria assessment.

In particular, in Italy it is needed an accurate planning of communication initiatives from the side of Institutions so as to manage a natural phenomenon that in the last years have been increasing, and represented by potentially toxic cyanobacteria blooms and by scum formation due to the presence of cyanobacteria in the internal waters, especially with reference to water for human consumption. In this context, the above said phenomena represent an emerging health risk that cannot be ignored, especially as far as the possible human exposure to different toxins (cyanotoxins) that those organisms can produce, thus causing a significant public alarm that Institutions cannot neglect.

Moreover, some critical points in the communicative process – such as the existence of incomplete and sometimes not agreed data and information deriving from activities carried out by several institutions, the different exposure modes and different toxic effects that cyanotoxins can cause, and the difficulty for people to recognize “guide values” meaning and reliability – outline the need to foster, first of all, an effective internal communication towards the scientific community and reference Institutions responsible for the surveillance and management of water resources and drinking water supply chain, as condition to ensure a consistent and transparent communication among all the figures involved in the issue.

This scenario therefore demands a communicative strategy focusing both on internal communication processes and on external communication ones addressed to multiple subjects involved with different roles, interests and perceptions.

5.1.1. Importance of internal communication

It has to be underlined that an effective communication within the scientific community and Institutions directly engaged in risk management, represents a crucial condition for a more efficient external communication, both in the stage where the phenomenon occurs, but not in an acute form, with a “0” alert level excluding an immediate health risk, and in the following stages of the surveillance and alert system until the emergency and post-emergency stage.

In particular, in emergency situations, the communicative process is more difficult inasmuch in a short period of time a series of very complex factors making risk management more complicated occur. Often, contrasting criteria and approaches are suggested both for phenomena assessment and management and several authorities must take action with different skills at healthcare or environmental level; moreover, interest groups often require different actions, contrasting from each other, in a context with a high perception of risks emphasized by contradictory positions of those who take part in the decision-making process.

Guidelines can significantly contribute to simplify the internal communication process, in that they not only offer tools for an adequate structure of prevention and emergency preparation measures, but also contribute to explain and match authorities roles and activities, organizations and interest groups involved in environmental surveillance systems, alert and emergency management as well as in the epidemiological surveillance system established following the health alert.

For this purpose, it is appropriate to highlight that when an increase of suspected intoxications cases occurs and it is necessary to establish an epidemiological surveillance system for the populations exposed, the implementation of communicative processes and synergies among Institutions, Structures and Authorities involved acquires a crucial importance.

An effective internal communication can in fact favour the integration of epidemiological and environmental data and can strengthen the interaction among all the subjects involved in system realization: institutions responsible for environmental protection (Environment and Territory Protection Agencies), structures responsible for the epidemiological surveillance (Epidemiological Monitoring, Epidemiology Units within Local Health Service Agencies), Local Health Authority, Local Government (Mayor supported by the Hygiene and Public Health Service) which has to establish limiting and restrictive measures for protecting the public health. Even information return to all the operators involved, to citizens and interest groups can be eased through effective internal communication processes.

Moreover, activities sharing may create favourable conditions for an appropriate communication with the population involved who must inevitably be informed on the initiatives carried out concerning the surveillance and its purpose.

It is important to underline that the effectiveness of internal communication is strengthen when Institutions and figures playing roles, functions and having responsibilities other than risk management, are essentially aimed at the exchange and debate and when they consider that the creation of crucial synergies for the realization and the conservation of territorial network is crucial. Often, the network involves key figures and Institutions non directly engaged in risk management, but strategically important for strengthening the communication with citizens, such as for example general medicine physicians, family paediatricians or schools.

Therefore, the integrated cooperation and information flow among all the professional figures of Institutions and Services involved in risk management, can, without any doubt, favour a coordinated planning of activities and priorities, simplify the communicative process with population, media and social subjects and allow a responsible and informed participation of community, including and enhancing plausible figures playing a significant and guidance role for the population (12).

5.2. Listening as condition for an effective communication

The information coming from the scientific world on risks related to the proliferation of potentially toxic cyanobacteria, confirms that the current controls regime ensures the safety of water for human consumption with a high level of protection for public health. Even in presence of a contamination exceeding the maximum acceptable value, some emergency response plans are implemented and use restrictions provisions adopted with particular attention to the groups at risk, envisaging the recourse to alternative supplies and the urgent implementation of adequate treatments to restore the compliance of the distributed waters.

Even the decision-making flow implemented in case of drinking water emergency is well defined in Legislative Decree 31/2001 and, in general, in the regional guidelines on decree application where the different roles are clearly identified.

However, the population involved is very worried and sometimes reveals scepticism and doubts against decisions made during risk or emergency situations, up to show, in certain circumstances, a complete absence of trust in Institutions.

The technical-scientific assessments, even when appropriately argued, are often underestimated or ignored and the "red algae", with reference to the widespread of *Plankthotrix rubescens* blooms in several Italian reservoirs, has to be identified as reason for the occurrence of serious intoxications and, based on exposure and latency time, of acute and chronic effects.

The worry for one's own health and for the entire family, as well as the fear for a possible harmful event, increases risk perception. The studies on factors influencing risk perception, highlight that this last is basically related to emotional factors to such an extent that a series of components corresponding to the "perceived offence" *(*outrage*)*, more than the real hazard that is the cause of the hazard itself (13), contribute to determine the perceived risk. Institutions must receive and "actively listen" people worries and be aware of offence "determinants" characterizing the perceived risk, so as to have greater opportunities to understand the origin of perception and be able to deal with it (14).

Listening together with empathy, skills and experience, honesty and frankness, dedication and engagement, represents one of the key factors on which reliability and people trust rely on (15).

More than 50% of communication reliability depends on the way in which people perceive the person who is in charge of communication. If people perceive empathy, listening and attention for their worries, their way of live and feel the risk, they will be more willing to listen and trust. If, on the contrary, the person in charge of communication is not reliable because he/she is "distant" from people and exclusively focused on his/her own information objectives, the trust level will be reduced and at the same time the emotional component of perception prevail on the cognitive one. Therefore the communication content, even if adequate and scientifically sound, it will not be perceived by people because passed through a relational process without empathy, not so focused on the identification of real informative needs of target, on its sensitivity and perception.

In those cases there is often the transformation of the communicative process in a conflict among contrasting positions where emotional reactions, sometimes even in a disordered way, prevail and the "focal issue", the topic, the situation object of the communicative exchange is lost of sight.

An active listening from Institutions side, enhances the relationship of trust and allows to take into consideration the risk perception of people that not always correspond to the one of experts (16).

5.2.1. Active listening and empathy

Listening and empathy are communicative competences, skills that can be learned in specific training contexts and that the operator can use in his professional relationship so as to enhance communication effectiveness.

Listening represents the first step in the professional relationship; it is based on empathy and on the other's point of view acceptance, on the creation of a positive relationship and of a non-judging mood (17). It is needed to show interest and attention to speaker's needs, to create a relationship of trust and cooperation, premise for a future coalition.

It is possible to listen putting oneself in the other's shoes, entering his reference scheme and trying to see the "world" with the other's eyes so as to understand the information from a rational and emotional point of view (thoughts, experience, emotions, significance) to understand his requests and needs.

To listen through empathy means, therefore, to open up to the other person, follow and deeply understand his worries and emotions, assuming the same point of view. This means to live for sometime "as if" you were the other, but without forgetting that it is just "as if". If there is no "as if" condition, then it is no more possible to talk of empathy but of identification.

Being empathetic does not mean confusing the two points of view even because often these two do not agreed. This rather means to recognize what belongs to oneself (what I would do, think, decide, feel in the same situation) and being able, at the same time, to suffocate its own point of view to "see the world with the eyes of the other person" so as to recognize and accept, without judgments and interpretations, what the other perceives, thinks, feels or decides and does in the same situation. Empathy is supported by distinction and not confusion.

In the professional relationship between expert operator and public, empathy contributes to maintain the roles separated, in fact, only through this distinction it is possible to recognize one's own sensitivity and face emotional reactions of public, thus avoiding defensive behaviours which are often the reason for conflicts and symmetrical escalations. Just through distinction it is possible to keep, in a transparent way, the appropriate distance from the public, to take part emotionally but without burning oneself. If the speaker perceives transparency, that is a correlation among emotions showed and those really felt, he opens up himself, otherwise he won't.

Therefore, to communicate in an empathic way means being congruent with what one thinks and feels and what is expressed through oral and non-oral communication. This means to be able not to judge, leaving for some time one's own values and perceptions for embracing the one of the other person "as if" were one's own world. It means avoiding directiveness, suggestions, interpretation.

But this is not enough, because to listen in an empathic way also means being able to give back this recognition and comprehension.

5.2.2. How to enable the empathic listening

Listening can be activated through the development of bidirectional communicative channels able to favour information flows and useful exchange so as to know public informative needs, its worries and for supporting the choices justifying the use of some interventions more than others.

Operators may arrange several communication media and tools to ease population or specific groups listening of risk perception: *vis a vis* interviews, telephone interviews, interviews to key figures, press and media analysis, focus group, public debates, in-person meeting.

The interaction between operator and person/s through interpersonal relationship generally represents the most effective way to implement the bidirectional exchange, so as to listen and deepen risk perception level, personal experience, information acquired, poor areas and to create the basis for a relationship of trust and cooperation.

Within the interpersonal context, it is possible to use a specific method called empathic mirroring which, through adequate communicative techniques, can ease the listening, thus favouring the focusing on the point of view of the other and on risk perception (18).

Crucial techniques of empathic mirroring are as follows: reformulation, clarification, ability in questions, use of first person messages ("I think that", "According to me").

"Reformulation" is a technique consisting in repeating what the other has just said, using the same words or rephrasing in a more concise way using other terms, without adding other concepts to the content. In this way, the operator may obtain a positive result from the other person, and this last knows of having been listened. One can wait the moment in which the other person has finished a sentence for intervening and resuming what has just been said. "You're telling me that…", "You mean that…", "In other words…", "Therefore, according to you…", "You think that…".

The person recognizing himself in the reformulation is sure of having been listened and understood and is therefore confident to further express himself and cooperate. He is also facilitated to stay focused on the issue and on how he faces it.

"Clarification" favours the self-understanding underlying, through oral communication, the emotions associated to content. This is clear both at oral and non-oral communication. "I can see in your eyes that you're worried"; "From your words I can feel you're uncertain about what I'm saying".

The "survey capability" is the ability in how to make questions, choosing the most adequate type based on interviews stages.

"Open ended questions" have to be preferred in the initial stage of the interview; they allow for a wider chance of answer, tend to extend and deepen the relationship, encourage opinions and thoughts exposition (*how, what he would like, could, may deepen, what he thinks).*

"Closed ended questions" are defined, they force to a sole specific answer, often stress an answer, limit the communication and make it more focused, demand only objectives facts and sometimes may seem restrictive and obstructing (*when?, where?, who?).*

Questions starting with "why" can be perceived by the person as accusingly, and should be avoided.

The use of first-person messages ("I think that", "According to me") make it easy to distinguish between what concerns the expert operator and what concerns the person, thus allowing to avoid conflicts and favouring a non-judging mood and an autonomous decision-making process.

5.2.3. Listening to communicate uncertainty

The empathic listening may favour the "uncertainty communication" process on the importance of which it is crucial to meditate especially in a context, such as cyanobacteria risks communication, where several criticalities occur and in which information are often incomplete and sometimes contrasting.

"Uncertainty communication" corresponds to processes communication and not to the outcomes, that is to the supported description of choices made or that will be made and the explanation underlying some decisions more than others. Declaring and supporting the uncertainty, it is possible to shorten the distance between a risk scientific-probabilistic assessment and a subjective personal assessment determined by the perception of risk which increases when the emotional level increases (19).

The communication on uncertainty comes from a basic choice: the need of Institutions to communicate, therefore it demands a strategy and planning of communicative process, favoured by the integrated participation and collaboration of institutions and systems involved at regional and national level. In fact, due to the fact that communication of uncertainty entails the choice of arguments and hypothesis that may explain, in a transparent way, to citizens, the reason for certain decisions more than others, it is crucial that the choice is shared among figures and organizations involved in the communication process. Sharing creates the conditions for the formulation of homogeneous, understandable messages, able to make people understanding the reason for certain choices, the consequences that they could entail, the reasons for which, at the moment, it is preferred to follow certain paths more than others.

It is important that people understand and are informed even in an uncertain way, declaring "what is known and what is unknown".

When people receive detailed explanations on hypothesis and/or paths chosen because considered, at the current level of knowledge, most likely or adequate, they have the chance to assess the situation with a greater serenity and "competence" and to arrange the choices within their life context. At the time of the emergency they will be more likely to be collaborative, willing to face difficult situations.

Moreover, when people understand and take part in the choices, they feel respected and trust Institutions which listen and understand worries of individuals and community and are responsible for a comprehensible information. If, on the other hand they feel "manipulated", mislead, they lose trust and it is more likely that they will respond with denial and panic or ignore the provisions in a situation of maximum emergency.

Sometimes Institutions avoid to make hypothesis or tend to reassure "Don't worry, be calm, everything is under control", they prefer "not to say", but "it is not possible to communicate" because even the silence is a communication form (20).

An information must always be given, reporting what has been done, what has been doing, what it is intended to do; transparency is basically the best choice.

5.3. Paying attention to risk perception

In cyanobacteria risks communication, the empathic listening represents, without any doubt, an important resource to understand the main worries of the population involved, especially as far as the most weak categories are concerned, such as, for example, children and pregnant women. People, in fact, tend to base their risk assessment not on the count of possible number of dead, injured people or damage to the environment, but on the perceived presence of specific characteristics of risks situations and on some properties perceived of risk source (20), such as, for example, the familiarity with risk, individual control, comprehension, effects on children, effects on future generations, personal engagement, uncertainty of scientific data, voluntary exposure, trust in Institutions (3).

In fact, due to the fact that people worries increase if the toxic effects of red algae blooms create risk conditions specifically for children and most sensitive persons such as pregnant women, the institutions involvement must necessarily be aimed, not only, at ensuring effective safety conditions in the different contexts, but also at taking into consideration this worry and favouring supported information flows and indications that may allow people, especially parents, to make functional choices for the health protection of their children and to trust reference Institutions.

Monitoring activity of potentially toxic cyanobacteria blooms, as well as the adoption of preventive and contrast interventions of favourable conditions for the occurrence of blooming, represent, without any doubt, important responses to community alert. These initiatives unfortunately have to be notified and shared with the subjects involved, including the population, through specific planned, coordinated and monitored communication initiatives from the side of relevant Institutions.

The scientific communication model and, consequently, the risk communication one to which reference must be made, is the participatory model based on the interactive exchange assessment among all the parties (22), concerning the attention to the emotional component of individual and collective perception (23), as well as the understanding of social and personal issues, that is crucial to make the scientific datum a useful knowledge for citizens.

5.4. Communication planning

For this reason, the Legislative Decree 31/2001, that is the implementation of Directive 98/83/EC concerning the quality of water for human consumption, envisages (art. 10) that the competent authorities supply consumers with information on the provisions adopted in case of exceeding the parameters stated in the Directive, taking however into consideration that the "algae" parameter is considered an accessory, that is its research is carried out according to the decision of the competent health authority.

Moreover, also Legislative Decree 116/2008, that is the implementation of Directive 2006/7/EC, concerning bathing water quality management, demands to local health authorities the creation of specific monitoring plans for the territory affected by cyanobacteria blooms for risk assessment, including information to the public. It is therefore possible to deduce, based on what has been stressed even in the current regulation, the importance of local health authorities role played in planning, activation and assessment of communication activities, including the "speaker" function especially in emergency stage, in particular with media.

To this it must be added that local health authorities must necessarily coordinate with and share the initiatives with other institutions and organizations part of the local reality and/or active at regional and national level that, in their turn, may, in different but organized stages, have the role of leaders in the communicative process (24):

- regional authorities;
- aqueducts operators;
- local administrations (Mayor);
- professional figures of local Health Institutions (ASL operators in hospital structures, general medicine physicians, family paediatricians);
- institutions, associations and important figures of the social context (citizens associations, schools, teachers, priests);
- other local institutions (ARPA);
- provincial structures and administrations;
- civil defence;
- epidemiological monitoring centre;
- local media;
- scientific institutions (ISS, University);
- Ministry of Public Health.

Most of the subjects mentioned above, play a significant role within the local community and have the possibility to interact with people in different space and time, even through differentiated relationships, making them in a favourable condition not only to collaborate to the correct, homogeneous, free from personal opinions and judgments information spreading, but also to receive indications on individuals perceptions and prevailing worries. This last aspect is crucial for the establishment of future communication initiatives.

5.5. Communication plan key points

Communication planning includes the development of the following different elements.

The identification of communicative process subject (*who communicates)*, that is a clear indication of the authority which launches, coordinates and follows the process.

The identification of communication target: general population and directly involved one, but alternatively, based on communicative process stage, even the other subjects involved (*who is the target).*

The definition of communication content (*what*): the communication content should be periodically updated according to the evidences acquired until that moment and should be adequate, homogeneous, clearly expressed, focused on target, notified through clear and unmistakable messages, even repeated, responding to target worries and showing empathy.

It is crucial that uncertainties and lack of information are appropriately considered and declared, from time to time, that evidences are clearly separated and supported by opinions and judgments, inasmuch this increases reliability of scientific Institutions and the relationship of trust and collaboration with reference institutional figures and related institutional structures (25).

In order to make citizens fully aware of data (in compliance with Legislative Decree 195/2005, implementation of Directive 2003/4/EC, concerning people access to environmental information), and to make these lasts a significant and relevant message for health, the communicative process must be developed *in itinere,* according to specific criteria and agreed conditions and the communication content has to be agreed, from time to time, among experts and local Administrations, based on collected data and available evidences, paying attention to individual and collective risk perception. To collect information on risk perception, it will be possible to use specific tools and methods: opinion leaders involvement, other local professional figures involvement, telephone interviews, analysis of information spread by media, focus group, face to face interview.

The identification of activities and communication tools (*how*): communication tools to be integrated, chosen from time to time according to the communicative objective, beneficiaries, liquidity available, human resources, timing and context: press release, interviews released to local or national media, internet websites, letters, brochures, telephone interview, *vis a vis* meeting, public debates, scientific publications, scientific conferences, interventions during meetings on the issue. Especially for those communication tools using the interpersonal relationship, it is important to pay attention not only to oral communication, but also to the non-oral (face expression, look, gestures and body movements, posture, mimicry) and para-oral one (volume, voice timbre and tone, rhythm, sighs, silence).

The identification of communication times (*when*): when it is the right moment to starting information flow, time dedicated to communication, operator times and individual times, time (historic-social stage) during which the communication is performed, starting and ending date of an informative campaign, availability of Institutions to quickly answer to media requests.

The definition of communication context (*where*): external context where the communicative exchange occurs and internal context involving the authority starting the communicative process, seen as personal internal space dedicated to relationship and listening.

The definition of communication objectives (*why*): objectives can be understood in a general way (communication is a right, a professional duty, it favours network creation, information exchange, integrated collaboration among institutions, institutional reliability realization, awareness and individual and collective empowerment enhancement), and in a specific way with reference to knowledge, behaviours and attitude. In fact, a communicative intervention, according to the tools used, to times and resources, can have as objective, the enhancement of knowledge, a behavioural change involving what people think or feel face to a specific phenomenon and, on a longer term, a behavioural change.

Here follows a useful scheme to organize communicative process planning:

Who activates the communicative process	**Target**	**Communicative objectives**	**Communication activities**	**Communication tools**	**Monitoring assessment**
………	……	………	………	………	………

From the operative point of view, in the initial stage, to start communication activities planning process, it is possible to envisage the creation of a multidisciplinary working group composed by experts of different Institutions, regional and municipal Structures and Administrations representatives, to establish, organize and share the communication strategy, to favour information flow and integrated cooperation among the subjects involved, to form “key” messages and, where applicable, to envisage in-depth training moments even on communicative skills.

5.6. Choice of communication tools

Scientific publications represent the main source of information on the issue, however, for the language used and the in depth level, they are addressed to a specific target of experts.

Therefore, it is needed a regular information to the healthcare figures, both inside and outside the territory, through differentiated methods and/or specific written material both at individual and group level (letter, e-mail, telephone call, meetings, newsletter, bulletins, LIS topical fact sheets, Land Information System).

In the emergency stages, to ease the contact with population residing in the municipalities involved, it can be useful to send a letter *ad personam* explaining with a simple language, in an abridged way and gradually, the situation, the initiatives launched, how they works, timings and, in particular, the advantages for each single person or families. This first contact can create the premises for additional chance of relationship with individuals, especially if the letter indicates telephone numbers and/or internet web sites of reference and the Service and/or Services to be contacted.

At the same time, the realization of a brochure and a poster to affix in those places identified in the communication plan (general practitioner's offices, Local Health Authority Service and all other places considered as appropriate for information spreading) can contribute to the spread of information describing context reality, focusing the attention on health risks and simultaneously supplying indications on how to avoid risks and on preventive actions to be implemented at individual and collective level.

The brochure must include a few significant and clear messages for the target to which is addressed, that is they have to be focused on their specific informative needs and on possible worries or doubts; the brochure must then describe the actions that public administrations and health institutions have adopted and/or will adopt in the near future and state a telephone number of reference with the name of Service promoting it. This for strengthen the fiduciary relationship. The language used must be simple, clear and understandable for the target to which it is addressed and must therefore avoid the use of technical terms that just a few persons can understand.

The brochure, including an appropriate graphic design making it attractive and readable, should indicatively consists of 4-6 pages and could be attached to possible letters sent to citizens or even delivered to the relevant operators within the reference Services or public meetings expressly organized.

The poster, on the contrary, must resume in short the contents of the brochure and outline the key words. The brochure and the poster represent adequate unidirectional media to reach a cognitive objective (to inform citizens), thus giving an answer to specific informative needs of target people.

However, their effectiveness can be strengthened if used together with other tailored bidirectional communicative interventions (*vis a vis* interview). The brochure informative value is, in fact, strengthened if handed to the operator at the end of a clarification interview. In this context, the brochure represents a communication tool used to strengthen the already exchanged information during the interview.

The brochure organization must include the development of the following points:

- short description of issue and risk (*what is it);*
- definition of its importance for health (*why it is important*);
- description of actions taken and/or that will be implemented by public administrations and health institutions (*what can be done*);
- method of contribution of each single citizen on how avoiding risks and on preventive actions to be implemented at individual and collective level (*the contribution of everyone is crucial).*

These communicative methods can be integrated through information campaigns mainly diffuse by local media. For this reason, institutional Press Agencies should always inform journalists, through press release, so as to provide them with reliable and not alarmist information and news. For this purpose, it is crucial that Institutions and professional figures responsible for the communication with the media, are aware of certain significant criteria and "rules" helping them to communicate and collaborate with the target (this aspect can be subject to specific training initiatives).

Moreover, *vis* a *vis* communicative interventions such as public debates on the issue or tailored meetings within specific institutional contexts, can strengthen messages effectiveness, especially if leaders are able to use specific communicative-relational and counselling skills, to be deepened, if necessary, in dedicated training contexts (26).

During the last years, in America, especially during emergency situations, new communication tools such as *social media* have often been used because they are already well established in the American society and can represent a way to rapidly inform public during a crisis. More than 40 millions of Americans use, more than once a day, tools such as Facebook, Twitter and other similar technologies which, by now, are widely spread even in our society. Certainly, *social media* cannot and must not replace the other communication tools, but if used in a strategic way, they can strengthen the current systems (27).

5.7. Conclusions

Based on the considerations made concerning communication on cyanobacteria risks, some crucial points related both to the communicative approach and to communication initiatives implementation, must be outlined.

First of all, communication on risk cannot be improvised, but has to be planned by reference Institutions and has to be leaded in a conscious and strategic way through specific skills.

Moreover, to communicate does not mean to inform in a unidirectional way or teach, but it means to allow a debate and the exchange among all the subjects involved in the risk situation with different roles and responsibilities. Therefore, a particular attention must be paid to internal communication processes among those directly responsible for risk management, including the key figures who can contribute to communication within the territory.

Communication on risks increases its effectiveness if based on risk perception listening, on what people think and feel against the risk and its consequences. Even if people have different interests and skills, they are able to reinterpret the information received, use them or reject them according to their own purposes and values.

Communicative strategy task is very different from the one to inform public or convince it of the goodness of choices made by technical figures or the decision-making authority; it is rather to launch a process stressing differences based on positions, recognize them, suggest and support choices concerning risks management, through the listening of public worries and avoiding to label the point of view contrasting the scientific assessment as distort perception or knowledge gap between public and experts.

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APPENDIX

Cyanobacteria: summary of current state of knowledge and specific recommendations

BOX 1

Environmental factors lying under cyanobacteria proliferation
The specific ecophysiological properties of various cyanobacteria, are very different and allow them to occupy several ecological niches in aquatic ecosystems, thus responding in a different way to environmental factors controlling their growth, and which are for example light intensity, temperature, water column stability and main nutrients, both in terms of availability and ratio among their concentrations. The interaction, among the effects of different environmental factors on cyanobacteria growth makes it difficult to predict blooms. In general terms it can be said that cyanobacteria prefer a quite high water temperature, a stable stratification, a high light intensity and high phosphorus concentrations, but there exist important exceptions to this generalization and, management strategies must include the knowledge of population taxonomic composition and the ecology of species involved.
Recommendations The understanding of environmental factors favouring cyanobacteria proliferation in a specific water body, it is crucial for risk management. However, it is important to have information on the environmental status of the water body before blooming, because the analysis carried out during and after blooming, are highly influenced by the bloom itself and can be misleading. A systematic collection of environmental parameters characterizing water bodies both during cyanobacteria blooms and in normal conditions, could be used to create a regional reference framework on the environment conditions favouring blooming in Mediterranean environments.
In-depth analysis section 1.1. *Rapporti ISTISAN* 11/35 Pt. 1

BOX 2

Toxic species and impact on water for human consumption in Italy
The abundant development of toxic planktonic cyanobacteria (bloom), is a common event in fresh, brackish and marine waters and it represents a serious environmental and health issue. Of almost 150 known cyanobacteria genres, more than 40 include species responsible for the production of cyanotoxins and, based on the capacity to produce these compounds, they are divided in producers and non-producers. At the current state of knowledge, the following algal species are considered potentially toxic: *Anabaenopsis milleri* *Anabaena perturbata* var. *tumida, A. bergii, A. lapponica* *Aphanizomenon ovalisporum, Aph. isatschenkoi, Aph. flos-aquae* *Aphanocapsa cumulus* *Cylindrospermopsis raciborskii* *Dolichospermum* (*Anabaena*) *circinale, D. flos-aquae, D. lemmermannii, D. viguieri, D. planctonicum, D. spiroides* *Hapalosiphon hibernicus* *Limnothrix redekei* *Lyngbya majuscula, L. wollei* *Microcystis aeruginosa, M. flos-aquae, M. viridis, M. wesenbergii, M. botrys* *Nodularia spumigena* *Oscillatoria tenuis*, *O. nigroviridis* *Planktothrix* sp. FP1, *P. agardhii*, *P. rubescens*, *P. formosa* *Phormidium formosum* *Raphidiopsis curvata, R. mediterranea* *Schizotrix calcicola* *Umezakia natans* In Italy, blooms causing the development of toxic species of cyanobacteria are related to species belonging to the genres *Microcystis*, *Planktothrix*, *Aphanizomenon*, *Dolichospermum* (ex *Anabaena*) and *Cylindrospermopsis*. To date, in literature, data on toxic species occurrence are available just for 61 lakes among the 500 ca. on the territory (not taking into consideration the minor lacustrine bodies), in 13 regions out of 20.
Recommendations At present, drinking water treatments in use are able to remove a great part of algal cells even if the sum of toxins content at intra- and extra-cellular level is generally considered, in drinking water, the most important indicator in the assessment of exposure risk to these toxins, as far as the water for human consumption is concerned. For cyanotoxins, the Italian regulation does not envisage, to date, reference values: if it is suspected a presence of toxic forms in concentrations representing a potential risk for human health, the relevant health authority will implement a monitoring plan in compliance with Legislative Decree 31/2001, art. 8 and related attachments, for optional parameters research. However, the World Health Organization (WHO) has established guide values for some toxic species already introduced by several nations in the regulations of their own legislations.
In-depth analysis: section 1.2. and 1.4. *Rapporti ISTISAN* 11/35 Pt. 1

BOX 3

Toxins associated to cyanobacteria
Toxins produced by cyanobacteria are numerous and different: many of them are discovered and many others must still be adequately characterized. According to their specific toxicity, they can be divided into three main groups: - the hepatotoxins, which damage the liver; - the neurotoxins, which act blocking the synaptic transmission; - the cytotoxins, which block the protein synthesis; - the dermatoxins: a great variety of toxins and lipopolysaccharides which cause skin and mucosal irritations. Among hepatotoxins, the most spread are microcystins and nodularins. Microcystins and nodularins have similar mechanisms of action: they act through the inhibition of PP1 and PP2A phosphatase enzymes, leading to a hyperphosphorylation of cytoskeletal proteins of hepatocytes. The consequence is a loss in hepatic sinusoids architecture that can lead to blood accumulation in the liver until death due to haemorrhagic shock. Among cytotoxins, cylindrospermopsin acts as inhibitor of the protein synthesis, causing tissues necrosis. Among cyanobacterial neurotoxins, we want to mention anatoxin-a and anatoxin-a(s). Anatoxin-a is a depolarizing agent pre- and post-synaptic: it binds with acetylcholine receptors blocking the nerve supply at CNS, PNS and neuromuscular junction level, with consequent muscular paralysis and asphyxia. Anatoxin-a(s) blocks the acetylcholinesterase activity of the peripheral nervous system: acetylcholine accumulation hyperpolarizes neurons, blocking the transmission of following signals.
Recommendations The absorption of toxins can occur through drinking water, waters for medical purposes, recreational waters, vegetables irrigate with water containing cyanotoxins, edible fish and clams which have accumulated microcystins, consumption of food supplement containing cyanobacteria. Therefore, it is important to: - avoid that toxins reach people through well known ingestion methods; - avoid the creation of conditions for the production, mainly, of microcystins, or to monitor, in particular, *M. aeruginosa* e *P. rubescens* blooms.
In-depth analysis: section 1.3. *Rapporti ISTISAN* 11/35 Pt. 1

BOX 4

Regulation and national reference values
With reference to the protection of surface water bodies associated to the presence of cyanobacteria and cyanotoxins, the Legislative Decree 152/2006 and amendments (Consolidated Law), with particular reference to the last amendment to Part III of Annex I included in Ministerial Decree 260/2010, establishes a "percentage parameter of cyanobacteria peculiar to eutrophic water" among the elements on which is based the calculus for the assessment of phytoplankton EQB for lacustrine types combinable in macrotype I1. For this reason and for all the biological, chemical and physicochemical parameters included in the Consolidate Law, there exist defined methods and timings for surveillance, operative and survey monitoring (Table 3.6 of the Decree). For the 3 monitoring methods, it is established a sampling annual cycle with a frequency of 6 times for phytoplankton and a bimonthly interval for physicochemical parameters that must in any case be associated to phytoplankton collection. For the surveillance and operative monitoring of all surface water bodies, a monthly interval, if necessary, has to be envisaged for the priority chemical substances in Table 1/A, while a quarterly interval, if necessary, for substances of Table 1/B. Reference should be made to the Decree for further details and exceptions. The Consolidated Law envisages the implementation of more intensive operative and/or surveillance monitoring, in terms of frequency and sampling sites, for chemical or biological elements showing high natural variability or caused by anthropic pressures. Closer frequencies are also envisaged for a survey monitoring to be carried out in certain situations "of alarm or for a preventive purpose for the assessment of health risk and information to public". Timings detected for the supporting monitoring are based on the supplied population and are similar to what is stressed in the Legislative Decree 31/2001. Concerning the drinking water protection, the Legislative Decree 31/2001 and amendments, establishes that the health Authorities can carry out regular monitoring on algae, including cyanobacteria, in water bodies for human consumption, in compliance with art. 4, clause 2 and art. 8(3) and in Annex I part C (optional parameters). The risk management system for the monitoring of cyanotoxins in water for human consumption, must be based on the respect, as stated by the Legislative Decree 31/2001 (art. 6), of the maximum acceptable value for cyanotoxins. In Italy, the maximum temporary acceptable value for the MC-LR in water for human consumption is equal to 1.0 µg/L referred to the total toxin content (intra- and extra- cellular). According to an extensively conservative approach for human health protection, with an overestimation in the toxicity assessment, in the worst appraisal approach, the 1.0 µg/L value must be referred to the sum of different MCs congeners concentrations in the sample, considered as equivalent of the MC-LR. For this purpose, there have to be found microcystin congeners elements through confirmation methods using the best analytical potential available and, as minimum criterion, the congeners elements for which analytical standards are now commercially available, [D-Asp^3]-MC-RR, MC-RR, MC-YR, [D-Asp^3]-MC-LR, MC-LR, MC-LA, MC-LY, MC-LF, MC-LW.
Recommendations - Adequate timings can be deduced for cyanobacteria monitoring in compliance with the Consolidated Law concerning the routine determination of phytoplankton in surveillance and operative monitoring of reservoirs (6 monitoring/year). Cyanotoxins research should be carried out in critical situations, deductible from biological, chemical and physicochemical parameters, as well as from previous occurrences. The Consolidate Law envisages, for all kinds of monitoring, closer intervals compared to those established for chemical and biological elements in case of natural seasonal variations or due to anthropic impact, as in the cyanobacteria and cyanotoxins case, according to the relevant Authority. A routine monitoring of cyanobacteria on a bimonthly/six-month basis, to be carried out or not together with the phytoplankton one based on different surface water bodies to be used for the drinking water supply, could ensure the protection of the resources involved and stress possible alarm situations. - In use restrictions adoption it has to be considered, in any case, what has been established in art. 10, clause 1 of the Legislative Decree 31/2001. - Cyanotoxins research must be extended to other compounds categories (e.g. cylindrospermopsin and anatoxin), in the presence of blooming (for an alert greater than 1) of species producing these toxins; the maximum acceptable value of cyanotoxins other than microcystins, can be fixed in compliance with the Legislative Decree 31/2001 and amendments, art. 11(1)(b). - The reference values established have to be considered as temporary and can be reviewed based on the update of knowledge status on risk assessment.
In-depth analysis section 2 *Rapporti ISTISAN* 11/35 Pt. 1

BOX 5

Water supply analysis
The assessment of water supply vulnerability concerning the risk of cyanotoxins presence in water for human consumption, demands an adequate evaluator training; the historical analysis of toxic cyanobacteria proliferations episodes and of the effectiveness of management measures implemented by the system has a particular significance for the vulnerability assessment of the system. The approach to be followed is based on WSPs and on ALF system described in sections 2 and 3 of this volume. All the information contained in *Rapporti ISTISAN* 11/35 Pt. 1, includes useful elements to assess the vulnerability of a water body against toxic cyanobacteria proliferations with a potential production of cyanotoxins and persistence of toxins (intra- or extra-cellular) in water for human distribution. In figure 2 of section 3 of this volume, there is a scheme of an appraisal process of system protection level, based on the analysis of risk prevention and mitigation measures concerning cyanotoxins presence in waters for final users.
Recommendations - The assessment of water system vulnerability concerning the risk of cyanotoxins presence in water for human consumption, demands an adequate evaluator training; the historical analysis of toxic cyanobacteria proliferations episodes and of the effectiveness of management measures implemented by the system has a particular significance for the vulnerability assessment of the system. - The study including the update on knowledge level related to cyanobacteria risk described in *Rapporti ISTISAN* 11/35 Pt. 1 and this volume, and deepened in the mentioned references, offers appropriate principles for the analysis purposes of the system. - Further in-depth analysis, on a case by case basis, can be shared with the “National group for the management of cyanobacteria risk in water for human consumption”.
In-depth analysis *Rapporti ISTISAN* 11/35 Pt. 1; section 1 of this volume.

BOX 6

Toxic cyanobacteria identification
Within an integrated system for quality control of water for human consumption, the observation of samples, the identification and count at microscope of toxic cyanobacteria, represent crucial information for the preliminary highlighting of potential risks and for carrying out assessment analysis and counter measures. The definite identification, at species level, of cyanobacteria is a complex operation needing the combination of molecular and morphological data. Modern classification systems, including this information, keep, in many cases, the definition of traditional genres, based on morphological characters visible in microscopy, and ecological ones. It follows that it is often possible to distinguish cyanobacteria within the samples, according to genres, provided that an adequate number of individuals is observed so as to take into consideration the significant morphological and morphometric instability due to environment conditions and growth stages. The use of molecular methods, especially the amplification of molecular genetic markers through PCR combined to sequencing of genes encoding for some of main cyanotoxins, represents, to date, a promising tools for the detection of presence and appraisal of toxins species abundance in waters. Although this approach allows to exceed the limits mainly associated to microscope observation (through which, as an aside, it is not possible to distinguish between toxic and non-toxic individuals belonging to the same species), further theoretical-practical progress are still needed for the approach to be adopted in routine analysis.
Recommendations - The identification and count at microscope of toxic cyanobacteria genres, are necessary to outline the risks for human health. - The definite identification, at species level, of cyanobacteria, needs the integration of molecular and morphological data; knowing these taxonomic criteria is a real need. - The microscope analysis is supported by molecular techniques allowing the specific attribution and quantification of genotypes producing toxins in samples.
In-depth analysis *Rapporti ISTISAN* 11/35 Pt. 1: section 3.1. and Appendix A

BOX 7

***Early warning* controls at catchment**
The analysis of algal pigments has been widely used to determine the structure of phytoplanktonic community in water samples as supplement or alternative to the microscopic count. The methods presented have to be considered as methods for the monitoring of cyanobacteria bloom in short times and on a large space scale. They offer algal biomass quantification in general and cyanobacterial quantification in particular, in terms of chlorophyll and/or algal pigments, with different levels of taxonomic definition according to the method used. The use of *in situ* fluorimetry and remote sensing for blooms monitoring, exploit the optical properties of some accessory pigments of the peripheral antenna complex of PS II (phycobilins and chlorophylls): both methods, comparing the *in situ* collected data with reference spectra, offer a quantification of cyanobacteria in terms of contribution to overall chlorophyll. The most developed fluorimetric probes allow to distinguish cyanobacteria with phycoerythrins from those with phycocyanins and to implement the tool with fluorescence spectra for each single species. In *remote sensing*, it is possible to use the majority of sensors available for a qualitative mapping and a wide coverage of the considered ecosystem: in the event of less massive blooms (concentration lower than 20 mg/m^3) and for quantitative mapping, it is necessary to use hyperspectral sensors which can separate and distinguish the electromagnetic spectrum region where cyanobacteria peculiar and exclusive response occurs. In the HPLC technique, the chance to separate specific carotenoids, allows to quantify cyanobacteria importance against other algal categories. For the analysis of algal pigments the chromatography technique is the most frequently chosen during high pressure liquid reversed-phase. Through the association of information on chlorophyll and toxins cellular content in cyanobacteria to these measures, it is possible to detect risk thresholds for different alert levels.
Recommendations - To consider the ecological behaviour of organisms to be monitored for an adequate interpretation of fluorimetric profiles. - To envisage a data collection on chemical-physical parameters useful for data calibration from remote sensing and to choose the appropriate sensor according on the outcomes one needs to obtain. - To use appropriate sampling and storage procedures. - To adopt analytical methods for determining algal pigments according to the resources available and to research purposes.
In-depth analysis section 3.2 *Rapporti ISTISAN* 11/35 Pt. 1

BOX 8

Cyanotoxins identification methods
In risk management related to the presence of cyanobacteria in water for human consumption, the screening surveys are used to quickly detect the presence and categories of toxins produced. Generally based on biological essays or, more used immunological or biochemical techniques, screening tests provide qualitative and/or semiquantitative information on analyte or analyte class presence surveyed with an adequate attention for the relevant toxicological level, the simplicity of execution and the high productivity. Immunoenzymatic screening methods for microcystins, indicatively tend to a limited overestimation of analytes amount. Confirmation methods based on chemical-physical properties determination, such as molecular weight, presence of chromophore or functional groups able to develop specific reactions, ensure the identification and quantification of different cyanotoxins classes and congeners elements with a high level of certainty. Coupled liquid chromatography with selective detectors, such as mass spectrometers, is the preferred system for cyanotoxins chemical determination. The information offered by screening and confirmation methods, can be used with a multi-stage analysis system, as envisaged by Water Safety Plan principles.
Recommendations - To use appropriate sampling and storage procedures, aimed at exposure assessment. To pay particular attention to sample storage and preparation procedures (e.g. filtration) to be used for determining the overall content (intracellular+extracellular) of toxins. The information deriving form analytical data, especially from reservoir monitoring, is determined by sample representativeness (position and depth compared to the catchment) as wells as by an overall assessment of the system according to the WSP and ALF scheme described in section 3 of this volume. - The adoption of analytical screening and confirmation methods for the determination of cyanotoxins, is determined by the available resources and research purposes: screening methods are useful in the emergency stage and routine monitoring; confirmation methods must support decision-making processes and risk assessment related to the reservoir and water system, according to WSP models, for example in a possible presence of several toxins congeners elements. - To adopt procedures ensuring and monitoring quality, such as for example, the use of an appropriate internal standard, if possible.
In-depth analysis *Rapporti ISTISAN* 11/35 Pt. 1: section 3.3. and Appendix B

BOX 9

Prevention measures: controls at catchment and on nutrients
An effective plan for monitoring and preventive/corrective measures is crucial for the management of natural water bodies potentially at risk due to unexpected cyanobacteria blooms. These measures can highly contribute to implement actions aimed at the reduction of bloom development risk. Moreover, knowing the interactions among nutrients amount, nitrogen and phosphorus relations and changes occurred over time in the use of territory, it is useful to identify and select the best corrective actions to be adopted. Prevention strategies are therefore very important for the overall management of algal bloom phenomena in surface water bodies. The external sources of nutrients to receiving waters are numerous and, often, the diffuse types, as those deriving from agriculture or atmosphere, are more difficult to manage compared to the punctual sources. The external nutrients inputs can be managed through land improvement operations in the draining area or a better management of the territory, through a reduction of fertilizers use, restrictions involving the access of farm animals to lakes and rivers, increasing of riparian areas and recovery of lagoons and forests in marginal areas. In any case, the concurrent reduction of both internal and external amount is crucial, but the removal of the internal amount is often a very complicated and heavy activity whose potential side effects on the environment are still not well known. The most compatible system, from the environment point of view, for implementing preventive measures, is the one of hydrogeological modelling which can reduce the effects related to the increase of water residence time, but it is often difficult to be implemented. In the event of numerous blooming of cyanobacteria, flow modifications techniques are very complicated and can be realistically carried out only in small size water bodies. In this case, the physical procedures seem to be the best environmental option to be adopted, but their use must be assessed on a case by case basis. Chemical procedures could be potentially very useful, but they still demand a lot of studies to assess their real impact on the environment. Therefore, an intense undertaking is needed to find sustainable technical solutions and to reduce the amount of nutrients and the unexpected occurrence of algae and cyanobacteria. Some of these activities can also contribute to mitigate the adverse effects associated to the progressive and ongoing global warming and to nutrients amount increase. In general terms, it has to be considered that the recovery of a water body in terms of eutrophication phenomena reduction, can be highly delayed over time compared to the reduction of phosphorous inputs due to external causes and, however, related to the historical series of nutrients inputs. This is especially true for water bodies characterized by wide extensions and subject to complex hydrodynamic phenomena and to solutes exchange between water and sediments.
Recommendations - For an effective management of fresh water resources, it is crucial to implement a regular monitoring providing timely indications on the possible occurrence of those conditions favouring, in the short term, cyanophytes algal bloom. - Both internal and external input sources must be reduced so as to favour a more rapid recovery of the eutrophised system. In any case, the effectiveness of actions focused on the reduction of internal inputs, is highly influenced by a parallel activity of external inputs reduction. - Until the implication related to the use of chemical methods will not be fully understood, the physical procedures, even if sometimes too complicated, represent the best option, especially for the protection of aquatic environment, even if they always have to be assessed on a case by case basis.
In-depth analysis section 4.1. *Rapporti ISTISAN* 11/35 Pt. 1

BOX 10

Removal of cyanobacteria and their metabolytes
In presence of potentially toxic algal blooms, an adequate strategy for the containment of total toxins level in distributed drinking water, is mainly based on the removal of cyanobacteria through interventions reducing cellular lysis phenomenon at the minimum. For this purpose, among the possible treatments described in literature (pre-oxidation, micro-straining, slow sand filtration or through a riverbank, coagulation and flocculation, flotation, membrane filtration), the best results have been obtained using filtration techniques such as the slow sand filtration, the micro and ultrafiltration. Being impossible to exclude the concurring presence of exocellular toxins deriving from cyanobacteria cellular wall breaking, it is anyway advisable to implement specific treatments for their removal, such as the adsorption on activated carbon, the reverse osmosis, chlorination and ozonation.
Recommendations - To implement treatments reducing cellular lysis to the minimum. - To optimize treatment conditions through preliminary tests on a pilot and/or laboratory scale.
In-depth analysis section 4.2. *Rapporti ISTISAN* 11/35 Pt. 1

BOX 11

Cyanotoxins degradation products
Cyanotoxins, following drinking water treatments, can generate potentially toxic process products. The identification and the toxicological potential of those process products must be taken into consideration in the WSP context. The oxidation with chlorine, that is actually the drinking water treatment process most used for the removal of cyanotoxins, can generate degradation compounds that have been studied to ensure an adequate management of the risk related to cyanotoxins exposure in water for human consumption. The scientific data available show that microcystins and cylindrospermopsin are effectively reduced by chlorine and form, respectively, 7 and 2 degradation products, characterized by spectrometric-mass techniques. These degradation products are associated to a low acute toxicity and to a reduced activity with bioessays on mice and protein-phosphatases. The anatoxin-a, on the contrary, shows a lacking reactivity against chlorination.
Recommendations - To date, the typical chlorination processes of water companies, the only deeply analysed, are not related to possible exposures to toxic products reacting to cyanotoxins potentially present in origin waters. - To regularly update the state of the art on treatments with a potential impact on human health.
In-depth analysis section 4.3. *Rapporti ISTISAN* 11/35 Pt. 1.

BOX 12

Emergency response plans
The state of emergency is represented through the evidence of a health risk for the consumer due to the presence in the water of cyanotoxins levels exceeding the maximum acceptable values. It has to be highlighted that, in many cases, the occurrence of a state of emergency is very rapid, even few days, and the time frame in which the emergency occurs usually involves a few weeks. It is therefore clear that a safety management of the emergency, the health, economical and social impact of these phenomena over consumers, are related to interventions promptness and actions suitability. Those aspects therefore need: - a previous preparation of the emergency plan; - a coordinated and planned engagement of all the parties involved during the emergency stage as far as suitable procedures are concerned; - a preparation of human resources, through an adequate training. Emergency plans are generally established by each operator of water system production for human consumption, thus representing different scenarios having effect on the water utility, such as for example, extreme climatic events or potential hostile actions against the system. The already existing plans can eventually be integrated with risk due to cyanotoxins, but they present some common elements such as the definition of individual tasks and responsibilities, creation of a “crisis unit” within companies, identification of tools and equipments for the emergency also through coordination among Companies of the same Province/Region, possibly coordinated by Prefectures.
Recommendations - To structure, in advance, emergency response plans through the integration of risk related to cyanotoxins within the emergency plans already existing in the water system. - To organize a technical roundtable which may grant, during an emergency stage, the presence of a multidisciplinary and coordinated expert team able to manage the different crisis stages at its best. - To define the supporting data and the decision-making process according to what is respectively stressed in section 4.2. and 4.3. of this volume.
In-depth analysis section 3 Volume 11/35 Pt. 2

BOX 13

Epidemiological surveillance system
The effects on human health related to cyanotoxins exposure have been extensively documented and the spreading of cyanobacteria bloom within water bodies need the creation of an epidemiological surveillance system over exposed populations. The reporting of healthcare events allows public health services to implement the required measures so as to avoid a further spreading of the disease. The surveillance system in question, must take into consideration the following elements: - environmental risk extent, through the monitoring of cyanobacteria presence in water basins of the relevant territory, through models such as the ALF one, recommended by the WHO; - possible exposure modes (oral or occasionally skin or inhalation exposure); - different pathologies related to the exposure, acute (skin and mucosal irritation, acute liver failure, respiratory arrest) and chronic (tumor and neurodegenerative disease occurrence). The assessment of effects related to cyanotoxins chronic exposure, mainly associated to their presence in water bodies, has been carried out in the past through ecological studies, comparing environmental monitoring data historical series and current data sources. The monitoring of the acute effects due to cyanotoxins exposure, can be carried out through prospective or retrospective cohort studies which offer the undeniable advantage of methodological accuracy. Both studies are relatively useful for public health, inasmuch they do not allow to gain timely information to support health authority decisions.
Recommendations - For monitoring the effects on human health of cyanobacteria blooms in water basins, it is recommended to launch a syndromic surveillance system. This system relies on the notification to the public health authority of cases with suspected cyanotoxins intoxication, through the dedicated web portal or paper information flow. Within this system the following has to be defined: - the surveillance reference territory; - the time *set* in which the surveillance must be carried out, taking into account the environmental monitoring data and the factors influencing the seasonality; - an interinstitutional cooperation system made official through technical roundtables organized by the various competent institutions and authorities (public health, environmental protection agencies, civil defence, basin authority, municipalities, etc.); - case definition developed according to the knowledge of toxicity mechanisms of cyanotoxins present in the relevant water resources; - a simple data transmission method (*web-based*); - method of information return to all the operators involved in the surveillance. - The identification of events frequency exceeding the expected threshold, established according to the available historical data, activates the health alert.
In-depth analysis section 4 Volume 11/35 Pt. 2

BOX 14

Risk information and communication
Within risk communication context, it is crucial that reference Institutions implement communication plans according to strategically established methods, thus avoiding to adopt interventions only in case of “emergency”. In Italy, blooming phenomenon of potentially toxic cyanobacteria in water for human consumption, represents an emerging health risk with social consequences that often Institutions cannot neglect. Prevention and surveillance activities, among which, for example, the monitoring of abnormal proliferations conditions, represent an important response from the point of view of an adequate risk management and, simultaneously, from the point of view of communication, they can be usefully shared with the subjects involved, including the population, especially in contexts characterized by a high level of interest and participation. The local health authorities play a crucial role in the planning, activation and assessment of communication activities, by sharing contents and actions with the other institutions and organizations at local level and/or active at regional and national level. These can include, among other, aqueducts operators, local administrations, professional figures of Health Institutions within the territory, Institutions, associations and significant figures of the social context, structures and administrations at regional and provincial level, local media, scientific institutions and other interested parties which can play, if organized, the role of communicative process leaders. An adequate communication plan must envisages several elements such as the identification of the communicator and the *target*, content definition, identification of media activities and times, definition of context and objectives. From the operative point of view, it is useful to create a multidisciplinary working group composed by experts of different institutions, to establish, organize and share the communication strategy, to favour information flow and integrated cooperation among the subjects involved, to form “key” messages and, where applicable, to envisage in-depth training actions. Effective communication tools include brochures and poster to be affixed in the places identified in the communication plan such as general practitioner's offices, Local Health Authority Service, pharmacies or specific informative campaigns mainly carried out by local media. *Vis a vis* communicative modes are represented by public debates on the issue or tailored meetings within specific institutional contexts. During the emergency stage, preferential communication tools are those allowing the direct contact with the residing population in the municipalities involved, such as for example, *ad personam* letters stressing through a simple and synthetic language the situation and the risk, the initiatives launched for its management, the methods of activities execution and expected effects, time and recommendations for each single person or families, public debates favouring population expression of worries or doubts and to strengthen the relationship of trust and the collaboration with the Authorities willing to listen and support possible decisions.
Recommendations - Communication plans from the initial stages and not only during emergencies. - Planning, by the local health authorities, of communication strategies based on dialog and active and integrated participation, with particular attention to the emotional aspect of individual and collective perception. - Coordination and sharing of activities between local health authorities and the other Institutions and organizations existing at local level and/or active at regional and national level. - Continuous update of operators involved in risk management and training on communicative skills so as they can be provided with appropriate communication tools for an effective communication with the various leaders.
In-depth analysis section 5 Volume 11/35 Pt. 2

GLOSSARY

In accordance to the above guidelines, the following definitions apply.

Absorption: Physical process in which a radiation hitting a material is absorbed and transformed in another kind of energy, often thermal energy.

Accuracy: The closeness of agreement between a test result and the accepted reference value. It is determined by determining trueness and precision.

Algorithm: A set of step-by-step simple instructions to obtain a specific result. Term commonly used in the IT sector (algorithm for automatic classification, algorithms for image processing, etc.).

Analyte: Substance that has to be detected, identified and/or quantified as well as the derivatives emerging during its analysis.

Apparent Optical Properties (AOP): Properties depending on the medium but also on the geometrical structure of light field.

Aquifer: One or more rock underground layers or other geological layers with a sufficient porosity and permeability so as to allow a significant flow of underground waters or the extraction of important amounts of them.

Artificial water body: A surface water body due to human activities.

Benthos: Ecological category including aquatic organisms, both freshwater and seawater, living on the bottom or on a solid substratum. It includes all the phanerogam algae, sessile and vagrant organisms, that is they live submerged in the mood with a protruding end.

Bioassay: Determination of the activity or quantity of biologically active material, through the measurement of its effects on living organisms.

Biochemical methods: Analysis methods using as basic determination principle the measurement of a substance biological activity through the detection of biochemical reactions involved.

C-18: Analytical columns for linked-phase liquid chromatography, composed of alkyl chains having 18 carbon atoms as stationary phase.

Capping: Creation of a surface coverage layer.

Carotenoids: Class of organic pigments found in photosynthetic organisms. There are more than 600 kinds of known carotenoids; they are usually divided into two classes: carotenes (which are hydrocarbons and therefore oxygen free) and xanthophylls (which on the contrary contain it). They are accessory pigments that during photosynthesis allow the absorption of different wavelengths compared to chlorophyll and protect this last from photooxidation.

Chlorophyll: It-s a green pigment, in chloroplast membranes of vegetable cells and in procaryote organisms performing the photosynthesis. Molecule structure is characterized by the presence of porphyrin heterocycle, within which there is an Mg-ion.

Coast: Border line between land and ocean, gulf, sea or big lake water.

Cohort study: Surveillance of healthcare events occurrence in a group experimenting a certain exposure to a risk factor in a determined time period. The cohort study allows to verify possible risk factors of a population through a confrontation of the different incidence of a certain healthcare event (for e.g. a disease due to cyanotoxins exposure), between the group exposed to the risk factor and the non-exposed group.

Confirmation methods: Methods providing complete or complementary information able to univocally determine the substance and, if necessary, quantify it at interest level.

Danger: A source of possible personal damage.

Direct analysis: Analyte determination carried out through the analysis of the sample without having recourse to extraction, purification and concentration techniques. The basic dilution or modification of pH is generally considered as direct analysis.

Domestic distribution network: Piping, fittings, appliances installed among taps usually used for the delivery of water for human consumption and the external distribution network. The delimitation between domestic distribution system and external network, hereinafter referred to as delivery point, is represented by the counter, unless otherwise stated in the supply contract.

Ecological study: Studies concerning the association between an independent variable (risk factor) and a dependent one (morbidity rate). The observation unit is composed of a population or community. Both the risk factor and the morbidity rate are measured.

Euphotic layer: Water layer in which can occur photosynthesis, usually intended as water layer in which the bright intensity is equal to or higher than 1% of the bright intensity reaching the lacustrine surface.

Eutrophication (from Greek word eutrophia): Condition of nutritive substances abundance in an environmental datum. It generally detects an overabundance of nitrates and phosphates in an aquatic environment.

Exactitude: Congruity between the average value obtained from a wide series of outcomes and an established reference value. The exactitude is generally expressed as distortion precision.

Extra-cellular content of toxins: Toxins content in the water sample to be attributed just to the fraction dissolved into the water, or free fraction.

Extraction: Procedure of isolation of analytes from the rest of the matrix. Often this process is combined with concentration enrichment of the analyte to be determined.

Fluorescence: Property of certain substances to re-emit the electromagnetic radiations received at a longer wavelength thus reducing the energy. An example of this process can be seen in all the materials containing fluorescent pigments, such as in highlighting pen and fluorescent varnish ink.

Full circulation period: Seasonal period, usually during winter, where temperature difference between epilimnion and hypolimnion is not sufficient to generate a difference in density so to prevent wind force to completely mix lake waters. In very deep lakes or with a reduced exposure to wind, circulation may just be partial, not involving the entire hypolimnion. It has to be noticed that there exist some lakes, called meromictics, where a deep layer is never mixed with surface waters.

Graphitized Carbon Black (GCB): Absorbing material used for chromatography.

Groundwater: All waters which lie below the surface of the ground in the saturation zone and in direct contact with the ground or subsoil.

Guide value: Value referred to the concentration of a chemical species or microbiological agent that, considering an effective consumption in the entire life cycle, does not entail any significant risk for human health.

HILIC (Hydrophilic Interaction Liquid Chromatography): Analytical columns for linked-phase liquid chromatography, including a polar stationary phase.

HLB (Hydrophilic Lypophilic Balanced): Absorbing material used for chromatography and extraction, composed of polymers with hydrophilic and lipophilic mixed characteristics.

Hypolimnion: The most deep and cold layer of a lake.

Immunological methods: Analysis methods using as basic determination principle an antigen-antibody interaction.

Inherent Optical Properties (IOP): Properties only depending on the medium and therefore independent from light conditions inside this last.

Inland waters: All running or stagnant surface waters, and all the groundwater within the perimeter which serves as a reference point to define the limits of the territorial waters.

Interferer: Matrix compound that can interfere with the extraction or the final determination of the analyte, causing false positives/negatives or quantitative analysis unreliability.

Internal standard/process standard: Substance not included in the sample, having physical-chemical properties similar to that of the analyte to be identified and that it is added to every sample as well as to each calibration solution.

Intra-cellular toxins content: Toxins content in the water sample to be attributed to the amount of fraction contained in the cells (intra-cellular fraction) and to the fraction dissolved in the water (extra-cellular fraction).

Ion trap: Mass spectrometer, can have tridimensional or linear configuration (LIT).

Ion-pairing: Reagent used to enhance mobile phase selectivity (organic eluent) in chromatographic analysis. These are molecules with a charge opposite to the one of the analyte of interest and an hydrophobic region related to the stationary phase and that, through a combination with eluent ions and balancing the charges of the stationary phase, enhances analytes separation.

Lake: A still and internal surface water body.

Level of interest: Substance or analyte concentration in a sample, that is significant to determine its compliance with legislation.

Limiting factor: Element present in a lower amount compared to organisms needs, and therefore limiting their growth.

LOAEL (Lowest Observed Adverse-Effect Level): The lowest amount level (exposure) in which an effect is observed.

Local authority: A form of cooperation between municipalities and provinces in compliance with art, 9, clause 2, law 5 January 1994, no 36 and, until the full functioning of water utilities, the public administration responsible for the service.

Matrix effect: Variation of the instrumental response due to the presence of organic and inorganic compounds in the sample to be analyzed and different from the analytes to be determined.

Matrix: The whole parts, including mutual chemical and physical properties, to be analyzed and differing from analytes.

Metalimnion or thermocline: Water layer in which occurs a temperature change based on a rapid gradient between the hot surface layer (*epilimnion*) and the underlying cold one (*hypolimnion*).

Minimum required performance limit: Minimum content of an analyte in the sample that must be detected and confirmed. This limit is aimed at the harmonization of methods analytical performance for those substances for which an allowed limit has not been established.

MIP (Molecular Imprinted Polymer): Absorbing material used for the extraction, composed of polymers with cavities or action sites specifically developed for analytes or class of analytes.

MS/quadrupole/quadrupole: Low-resolution quadruple mass spectrometer, that can be single or triple.

NOAEL (No Observed Adverse Effect Level): The highest level for which no significant negative effects have been statistically occurred, based on frequency or seriousness, for the population subject to tests, compared with an appropriate population of reference.

NOEL (No Observed Effect Level): The highest level for which no significant effects have been statistically occurred, for the population subject to tests, compared with an appropriate population of reference.

Operator: The water utilities operator, in compliance with art. 2, clause 1, recital o-bis) of the Legislative Decree 11 May 1999, no 152 and amendments, as well as anyone who provides the water to third parties through autonomous water systems or fix or movable tanks.

Orbitrap™: High-resolution mass spectrometer.

Overall toxins content: Toxins content in the water sample to be attributed to the amount of fraction contained in the cells (intra-cellular fraction) and to the fraction dissolved in the water (extra-cellular fraction).

PAR radiometer: Tool for measuring the photosynthetically active radiation intensity, which is the bright radiation at wavelengths usable by chlorophylls. Conventionally wavelengths between 400 and 700 nm are taken into consideration.

Phycobilins: Water soluble pigments in the photosynthetic membranes of cyanobacteria and in red algae and cryptophyte chloroplasts, where they play the role of accessory pigments. They are found in photosynthetic membranes associated to proteins. They are divided in red phycoerythrins and in blue phycocyanins and allophycocyanins.

Phytoplankton: Group of autotrophic organisms in the plankton and with photosynthetic ability. They are cyanobacteria and microalgae, or rather microorganisms able to synthesize the organic substance from dissolved inorganic substances, using the solar radiation as energy source.

Pollutant: Any polluting substances, especially those listed in annex VIII to Directive 2000/60/EC.

Pollution: The direct or in direct introduction, due to human activities, of substances or heat in the air, in the water or in the soil, that can be harmful for human health or the quality of aquatic or terrestrial ecosystems depending from aquatic ecosystems, thus disturbing, damaging or deteriorating the recreational values or other proper environmental exploitation.

Precision: Congruity among results of independent tests obtained in agreed conditions (pre-established). The precision of the measurement is usually expressed in terms of inaccuracy and considered as a standard deviation of test result. A lower precision is determined by a wider standard deviation.

Pretreatment: Operations preliminary to the real sample treatment consisting in extraction and purification. Pretreatment examples: freezing, drying, filtration and pH modification.

Pseudo-molecular ion: Ion detected in mass spectrometry and associated to the molecular mass of the compound. Usually they are molecular ions with addition or loss of a proton - $[M+H]^+$ or $[M-H]^-$ kind.

Purification: Process for the removal of matrix compounds from the extract containing the analytes to be determined.

Qualitative method: Analytical method which identifies a substance based on its chemical, biological or physical properties.

Quantitative method: Analytical method which determines substance mass quantity or fraction so as to express it as numeric value of appropriate measure units.

Reactive phosphorus: Concentration of phosphorous detected through spectrophotometric measurement (molybdate method) without oxidation of the sample.

Redfield report: Optimal theoretical report among carbon, phosphorous and nitrogen for algal growth.

Reflection: Process according to which the radiation hitting a material surface is returned, completely or partially, in the same means by the interface dividing the two means considered without the occurrence of radiation frequency change.

Reproducibility: Accuracy in reproducibility conditions, or rather conditions through which tests results can be obtained using the same method on identical test elements in different laboratories with different operators using different appliances.

Risk analysis: Process including three interconnected components: assessment, management and risk communication.

Risk assessment: Scientific process composed of four stages: danger detection, danger characterization, assessment of risk exposure and characterization of risk.

Risk management: Process, different from risk assessment, consisting in the examination of alternative interventions through dialog with the parties involved, taking into consideration risk assessment and other relevant factors and, where necessary, making appropriate prevention and monitoring choices.

Risk: Function of likelihood and seriousness of a harmful effect for the health, due to the presence of a danger.

River basin: The area of land from which all surface run-off flows through a sequence of streams, rivers and, possibly, lakes into the sea at a single river mouth, estuary or delta.

River: An internal water body mainly flowing on surface but that can be partially underground.

Robustness: Measurement of an analytical procedure ability of not being altered by small (voluntary) variations of parameters that, beforehand, can predictably influence results. It estimates the reliability of the procedure during routine work.

Screening method: Method used to detect the presence of a substance or of a class of substances at interest level. Those methods allow to rapidly analyze a high number of samples and are used to examine a great number of samples searching for potential non-compliant outcomes. They are specifically designed for avoiding false compliance results.

Secchi disc: A white and black colour disc, generally with a 30 cm to 1 meter diameter used for the assessment of lacustrine and marine water transparency based on the depth at which it becomes invisible.

Selectivity: Analytical technique ability of not be influenced by the presence of interferers or other components other than the analyte studied. Sometimes, the term specificity is used to express the same property. An analytical method selectivity against two different analytes, can be expressed through their sensitivity ratio.

Sensor: Any devices collecting electromagnetic energy coming from the scene and transforming it in an electric signal including information on the scene itself. This term is also used, for assimilation, for the camera.

Senstivity: Ratio between the signal variation (response) and the stimulus variation (e.g. concentration) producing it. In the event of calibration diagram, sensitivity is the gradient of calibration function: it can be constant or not.

SPE (Solid Phase Extraction): Extraction technique based on solid-liquid chromatography which uses several absorbent materials usually contained in cartridges.

Specificity: Method ability of distinguish between the analyte to be measured and the other substances. This peculiarity is mainly a function of the measurement technique described, but it can vary according to compound class or matrix.

Spectral response: Response of a material as function of incident electromagnetic energy wavelength especially in terms of measurable energy emitted or reflected.

Spectroradiometer: Spectrometer able to measure the radiant flux density of emission or absorption lines of source, over the entire spectrum. The spectroradiometer generates simultaneous scene images, in very close consecutive spectral bands. To each pixel is therefore associated a continuous spectrum that can be used to identify the peculiarity of light source and not some radiance values.

Sub-basin: The territory in which all the surface waters flow through a series of streams, rivers and possibly lakes flowing in a specific point of a stream (usually a lake or the confluence of a river).

Summer stratification: Seasonal period during which temperature difference between surface hot waters and the cold ones is sufficient to keep a stable stratification.

Surface water body: A distinct and significant element of surface waters, such as a lake, an artificial basin, a stream, river or canal, part of a stream, river or canal, transitional waters and coastal waters.

Surface water: Inland waters, with the exception of groundwater, transitional waters and coastal waters, except with respect to the chemical status, including territorial waters.

Syndromic surveillance: Epidemiological surveillance systems based not on disease diagnosis but on the presence of a series of signs and symptoms establishing a syndrome. This kind of surveillance is less specific but at the same time is more sensitive because it takes into consideration all uncertain diagnosis cases that otherwise would not be communicated by physicians. Those systems are therefore the objective to prematurely identify potential menaces for public health, so as to implement a rapid response to reduce morbidity and mortality, and can usefully integrate information coming from surveillance of already existing disease in the majority of countries.

TDI (Tolerable Daily Intake): Amount that can be swallowed every day for the entire life cycle without being dangerous for life.

Thermal stratification: Development within a lake, of two layers with different density due to different temperatures. In lakes sufficiently deep of temperate areas, usually there is a summer stratification, with the highest layer (epilimnion) hotter than the deep waters (hypolimnion). In the most cold areas can occur a winter inverse stratification, with surface waters at a lower temperature compared to the deep waters that will show a water density maximum temperature (almost 4°C).

Total phosphorous: Concentration of phosphorous contained in the water, measured through sample oxidation and following spectrophotometric analysis. It also includes a considerable amount of dissolved organic phosphorous and the one contained in the organisms within the sample.

Underground water body: A volume of underground waters contained in one or more aquifers.

Water body highly modified: A surface water body whose nature, following physical alterations due to human activities, is basically modified, as resulting from the designation provided by the member State according to Directive 2000/60/EC annex II.

Water for human consumption: Here follows the different categories: 1) treated or untreated waters for human consumption, for beverage and food preparation or for other domestic use, notwithstanding their origin, both delivered through a distribution network, tanks, bottles or containers; 2)water used in a food company for the manufacturing, treatment, storage or commercialization of products and substances for human consumption, excluding those in compliance with Legislative Decree 31/2001 art. 11, clause 1, recital e), whose quality cannot have consequences on final food product healthiness.

Water utilities: All the services provided to families, public institutions or any economic activities.

Zooplankton: Together with phytoplankton and bacterioplankton represents one of the three parts of plankton and it is composed of animal organisms, which are moved by the stream. Zooplankton is usually divided into three range based on individual dimensions: mesozooplankton, macrozooplankton, megaplankton.

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