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Review and analysis of occurrence, exposure and toxicity of cyanobacteria toxins in food

Emanuela Testai, Franca Buratti,, Enzo Funari, Maura Manganelli, Vichi Susanna, Nathalie Arnich, Ronel Biré, Valérie Fessard, Ateliana Sialehaamoa

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EXTERNAL SCIENTIFIC REPORT

APPROVED: 10 February 2016 PUBLISHED: 22 March 2016

Review and analysis of occurrence, exposure and toxicity of cyanobacteria toxins in food

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Abstract

This report presents the extensive literature search conducted on 1) the occurrence of different cyanotoxins in food matrices; 2) the analytical methods for their detection; 3) their toxicological profile; 3) the environmental factors affecting toxicity of cyanobacterial population and 4) the combined effects of mixtures of cyanotoxins and other chemicals. It also includes a review of guidelines values or health-alert levels for cyanotoxins in food (or drinking water) adopted world-wide. The methodological aspects and the queries used in the extensive literature search, the collection and screening of retrieved papers and the inventory are briefly described in the report; all details are available in 3 supplementary appendices to this report. The analysis of collected papers indicated that most of them are focused on a single microcystin (MC) variant (MC-LR) out of the almost 100 MC known. Many studies on occurrence are affected by limited quality, due to analytical drawbacks in the detection methods and were not considered in the exposure assessment. Toxicity studies useful for the derivation of health based reference values are few, being many of them carried out using i.p. injection, which is poorly representative of actual human exposure. In addition, those toxicological studies carried out with poorly characterised cyanobacterial extracts or focused on single parameters, using a single dose, devoted to elucidation of mechanism of action, reporting qualitative description of effects were not used for data extraction. The relevant exposure scenarios are also described, although being the available data on exposure very limited, no definite conclusion on the health risks for the exposed population could be drawn. However, the possibility of risky exposure is evidenced for fish and shell-fish consumers and for blue-green algae supplements (BGAS) as well in relation to MC contamination. Finally, many data gaps were identified.

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Key words: Cyanobacteria, cyanotoxins, occurrence, toxicity, exposure, risk assessment, data gaps.

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Summary

This report presents the results of the extensive literature search (ELS) conducted on 1) the occurrence of different cyanotoxins in food matrices (including: Microcystins, Nodularins, Cylindrospermopsins, Anatoxin-a, Homoanatoxin-a, Anatoxin-a(S), BMAA, Saxitoxins, Lipopolysaccharides toxins, Aplysiatoxins, Lyngbyatoxins, Palytoxin-group toxins and/or ciguatoxin like toxins); 2) the analytical methods for their detection; 3) their toxicological profile; 3) the environmental factors affecting toxicity of cyanobacterial population and 4) the combined effects of mixtures involving cyanotoxins. It also includes a review of guidelines values or health-alert levels for cyanotoxins in food (or drinking water) adopted world-wide.
On the basis of the analysis of the ELS results data were reported to address:

1) Exposure assessment, reporting data on occurrence and analytical methods and the identification of the exposure scenarios

2) Hazard identification and characterisation, reporting data on the toxicological profile of cyanotoxins, information on mixture toxicity and on the environmental factors affecting toxicity of a bloom, and the derivation of health-based guidance values (HBGV), when possible.

3) Eventual risk characterisation, by comparing HBGV with exposure drawing conclusions whenever possible.

Data gaps, recommenadtions as well as any other issue considered relevant for the EFSA risk assessment activities are also reported.

This Final Report is supplemented by 3 Appendices:

1) Appendix A included the information source forms, reporting the queries used in the extensive literature search on the different databases (for the different tasks: occurrence in food, analytical methods, toxicity, environmental factors affecting toxicity and mixture toxicity involving cyanotoxins), the detailed information on the number of initially retrieved papers, the included/excluded papers during the screening phases, and of those papers from which data have been taken and included in the inventory;
2) Appendix B corresponds to the full list of references in an EndNote™ Library, provided as a set of files corresponding to different cyanotoxins; within each file the papers have been further subdivided depending on the matrix in the case of occurrence, and on the species (i.e. fish or mammals) or the toxicity endpoint for the toxicological profile definition; separate EndNote™ files are provided for the environmental factors affecting cyanobacterial toxicity and for the mixture toxicity;
3) Appendix C is the Data Inventory (in tabular format, as a collection of MS Excel spreadsheets, a subset for each cyanotoxin, further subdivided depending on the matrix in the case of occurrence, and in vitro, in vivo and epidemiological studies for toxicity) containing the data extracted from the relevant papers, which can be can be downloaded from the EFSA website in a Microsoft Excel format together with this report.

Here a summary of the methodological aspects (described in details in the previous Deliverables) is given together with a narrative text, describing the results for the analysis of occurrence data exposure (including analytical methods) as well as for the toxicological profile data and the environmental factors affecting toxicity of cyanobacterial population. The analysis indicated that the great majority of the studies are focused on microcystins (MC) and specifically on a single MC variant (MC-LR) out of the almost 100 MC known; only a limited percentage is devoted to the sum of the other toxins and for this reason in some cases results have been described for MCs and for 'Toxins other than MC'.
Regarding occurrence, many papers were affected by limited quality and therefore excluded. However, even for those papers included in the inventory a further quality check has been included, integrating information on the reliability of the analytical methods used. The availability of reliable analytical method is one of the major issues considering the topic of cyanotoxins contaminations of

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complex matrix, as food items are. Indeed, while methods have been validated for toxins detection in water, the situation is still problematic when complex matrices (e.g. food items and other biological samples) are concerned. Moreover, the detection technique used is only the end of the analytical chain; sample treatment is equally if not more important as it will affect the final result.
The quantitative extraction of the toxin seems to be very difficult to achieve when dealing with complex matrices such as blue-green algae supplements (BGAS), plants and aquatic organisms (fish, molluscs, crustaceans etc.). In addition some toxins such as MCs and β-N-methylamino-L-alanine (BMAA) are known to exist as free toxins or bound to proteins; in such circumstances it is important to use the adequate extraction procedure depending on the objective that has been set (i.e. taking into account the free or total toxin in the study). In the absence of an indication about the detection of free, bound or total toxin form, results cannot be compared.
The use of cleanup steps in the analysis of cyanotoxins has been largely reported with the aim of reducing the matrix load and thereby the interferences, but they are also likely to reduce the toxin content through losses during the process. Unfortunately, the recovery rates of such procedures are not always reported. Regarding the detection techniques used for the analysis of cyanotoxins, they can be classified as chemical (capillary electrophoresis (CE), gas chromatography (GC) or liquid chromatography (LC) coupled with ultraviolet-visible (UV) spectroscopy, fluorescence detection (FLD) or mass spectrometry (MS)) or biological (bioassays, immunoassays, cytotoxicity assays, receptor binding assays).
LC-MS technique is frequently reported as detection method. Yet, it is prone to matrix effects, responsible for inaccuracy due to ion enhancement or suppression especially in ESI mode: these matrix effects are not systematically assessed. If LC-MS is very specific, especially in high resolution, this technique is used for targeted analyses, meaning that what is detected is what has been looked for, and there is a need for an analytical standard for an appropriate quantification. On the side of biological techniques, immunological methods (ELISA, SPR) are only semiquantitative methods: the use of monoclonal/polyclonal antibodies directed against a toxin/toxin family helps recognizing analogues of the same family (even unknown ones, without the need to have any analytical standard), but the cross-reactivity of the antibody towards the toxin analogues can greatly vary among variants, making any quantification difficult. In addition ELISA is also hampered by matrix effects. Other semi-quantitative methods such as cytotoxicity assays, enzymatic inhibition and receptor binding assays have been reported for the analysis of cyanotoxins in complex food matrices with occasionally characterization data to assess their performance.
Furthermore, the observations made are very toxin/matrix dependent and may be different for another toxin/matrix couple.
On this basis the papers on occurrence included in the inventory were further screened based on a quality score system (from 1 to 4) taking into account the quality of the analytical method used: those results obtained with an unreliable quantification method (not validated, not considering possible matrix effects, not reporting any recovery information) were not further considered for the elaboration of the exposure scenarios.
When considering the reliability of the analytical method, only few data remain on MC occurrence from field studies expressed as fresh weight (and only a small percentage refers to European data). Some laboratory studies on fish and shell-fish are also available, but they generally use high unrealistic exposure conditions and were not considered for further elaboration in the exposure scenarios. In addition, only data expressed as fresh weight were considered as first choice, since, although a conversion factor could be used to obtain fresh weight from dry weight samples, this extrapolation is affected by a high degree of uncertainty, mainly due to lack of information on the procedure to obtain dry weight.
The data collected show the broad prevalence of MC in aquatic edible species from European and non-European countries (North America, South America, African countries, Asian countries) confirming that accumulation of microcystins is a global concern. Liver and viscera in fish have been reported to have higher levels than muscle tissue, but no relationship can be found, due to spotted results in different species, that increase variability. In addition, the available data seem not to indicate a clear and direct relation between the fish species with different feeding strategies and MCs concentrations measured in their tissues. Cooking (boiling in water, microwave) may alter MCs concentration,

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although very contrasting results are available, showing various degree of increase, no effects or even a decrease. The few results available on livestock rely on a very limited number of animals (2 or 4 depending of the few studies available), further research is needed before drawing a conclusion regarding the possible transfer of MCs to the milk or the meat (with a more accurate analytical method and taking into account the bound-MCs). Only one study measured MCs in the muscle of wild birds and game hunting. Considering crops, when the reliability of the analytical method was taken into account, only one study remains from field studies regarding water chestnut from China but it is not sure if the results are expressed as fresh weight or dry weight (lyophilized samples). No data remain for European countries. Regarding food supplements only 5 studies (2 from EU Countries: range 0.86-5.20 µg MCs/g) passes the quality check for the analytical method used for *A. flos-aquae* products.

The toxins nodularins, BMAA, cylindrospermopsins and anatoxins have been the most investigated among the group 'Toxin other than MC'. However, numerous data come from laboratory studies where accumulation in different types of organisms has been tested. Toxin occurrence data in animals or crops through field studies were very limited, when quality criteria were applied. No study was included in the inventory regarding ciguatoxin (CTX), palytoxin (PlTX), Lyngbya toxin and its congeners. Few studies have been conducted on food supplements derived from *Spirulina* and *Aphanizomenon* only for anatoxins and cylindrospermopsin showing some variability in their results.

Regarding the toxicological profile, the situation is similar: the number of retrieved papers was much higher than expected, but most of them were on MCs, particularly on one single congener (MC-LR).The paucity of papers addressing the presence of cyanotoxins other than MCs in freshwater environments, in food items or measuring their toxicity leaves the mistaken impression they are of minor concern in relation to human health. The low detection frequency may partially reflect the low method sensitivity and the scant availability of certified standards; the need to have analytical validated methods in complex matrices is absolutely a priority in order to have reliable data on exposure, understanding if further data on their toxicity is needed.

Priority for data extraction, as described in the Methodology section, has been given to those *in vivo* studies useful for the derivation of health based reference values, which are unfortunately not many. Among the retrieved papers there are many *in vitro* studies and a large amount of records on cellular/molecular target related to phosphatase inhibition and oxidative stress induction (sometimes redundant and repetitive), especially after a single acute exposure. A large amount of records on fish are also available, relevant considering their presence in water, but of limited use for human health evaluation.

The more recent studies on MC are focused mainly on reproductive toxicity and organ toxicity other than liver (e.g. thyroid), in some cases with administration of a single dose (never following any guideline test method), and generally using the i.p. route of exposure, which is poorly representative of the actual human exposure. In addition on the basis of the available data on MC-LR, i.p. exposure has marked differences (up to 30 fold) with respect to acute toxicity when compared to the oral route (likely due to kinetic differences). Some recent papers reported the use of OMICs technologies for MoA studies, many of which on different fish species.

One limitation to toxicity studies conduction could be the limited availability of the toxins; for this reason studies are often carried out with poorly characterised cyanobacterial extracts (with a number of confounding factors) and therefore are not useful for the risk assessment. Indeed the available data indicate that components other than cyanotoxins are responsible for some toxicity and contribute to increase the toxicity of the extracts when compared to 'pure' toxins. This is true for MC variants, but also for the other cyanotoxins for which the data base is much more limited.

For some MC variants (a small % with respect to the known ones) it was possible to compare the acute toxicity having some data after i.p. injection: a single aminoacid difference (MC-LR vs MC-RR) can result in a 10 fold difference in the i.p. LD_{50}. A TEF (Toxicity Equivalent Factor) approach has been suggested, but considering the marked difference between the oral and the i.p. route and the possible kinetic differences (involving the active transport mediated by OATPs, distribution, metabolism via

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GSH-conjugation) among hydrophilic (as MC-LR is) and more lipophilic (e.g. MC-LF) variants, information is likely to be too preliminary to be applied.
For MC-LR a provisional reference value for subchronic exposure (0.4 µg/kg bw/day) could be derived using the NOAEL from a subchronic toxicity study as PoD; on the same basis a provisional TDI of 0.04 µg/kg bw/day can be derived as well (as WHO did to obtain a Guidance Value for drinking water), although with a high degree of uncertainty. The WHO provisional TDI value for MCs, which has hitherto represented the only pragmatic, quantitative approach to the management of risks associated with exposure to this class of cyanotoxins, has some limitations, mainly due to the scarcity of information available, not exhaustive in either kinetic or dynamic respects. It can be discussed whether or not an additional safety factor should be included to account for the tumour promoting activity of MC, which on the other hand has a thresholded mechanism, related to the inhibition of hepatic protein phosphatases. In addition, the evaluation carried out for MC-LR has been suggested to be extended to all the others, expressing it as MC-LR equivalents, but the validity of extrapolation from the acute toxicity ranking obtained via i.p. injection to other routes of exposure, and most importantly among MC congeners, to chronic toxicity remains to be demonstrated. However, the WHO Guidance Value (GV) for drinking water is the one to which most of the recommendations/regulation and/or alert values make reference (as described in the appropriate section of this document).
From MC-LR toxicological data it is also possible to estimate safe concentrations with regard to the acute risk following oral exposure: an acute NOAEL of 25 µg/kg bw, the highest dose at which no hepatic effects are observed can be derived from i.p. injection studies in mice, but again the uncertainty is high, especially considering the steepness of the dose-response curve.

Regarding the databases for anatoxins and cylindrospermopsin on repeated, long-term oral toxicity, they have not been deemed adequate to derive any TDIs; indeed no other provisional GVs for cyanotoxins have been defined by the WHO so far and only a tentative health based value derivation has been proposed for ATX, and used for setting limits or guidelines in some Countries, but the levels of uncertainty is even higher than for MC. Studies characterized by repeated exposure duration and oral route show no 'significant' effect at the highest doses tested, and were therefore not robust enough to derive a TDI value. The use of an unbound subchronic NOAEL (510 µg/kg bw/day) and an assessment factor of 1000 (10 for the interspecies variability, 10 for the intraspecies variability and 10 for the scant data base), will lead to a 'minimum Tolerable Daily Intake' (mTDI) of 0.5 µg/kg bw/day. However, other proposals, discussed in this report, have also been formulated by some authors and found in the literature, using a different PoD.

For those toxins already evaluated by EFSA (EFSA Journal, 2009a, b, 2010), some new data are available which could give indications for an update. However, data are still limited and the derivation of health based values would still be affected by a high degree of uncertainty.

Two examples in this respect are STXs and PlTX. The extensive literature search from 2009 onwards pointed out that some new relevant information have been provided by recent studies on STX-group, although several aspects still remain insufficient for a complete characterization of their toxicological profile. The paper by Munday et al. (2013) tackled the problem of the use of TEF, currently applied following the EFSA opinion (2009). TEF is based on the Mouse BioAssay (MBA) method and, knowing the specific activity of STX itself, permit the expression of the activities of specific derivatives in terms of STX equivalents. It was already pointed out by EFSA that this approach implies a large number of uncertainties: firstly, in the MBA STXs are administered by i.p. injection, which is a non-relevant route of exposure to saxitoxins for humans, being the oral route the most significant one; secondly, no correlation could be drawn between the specific activities determined in the MBA bioassay and the median lethal doses (LD_{50}) of the test compounds; thirdly, there is no evidence that the shape of the curve describing the relationship between dose and death time for all the STX derivatives is the same as that for STX itself. When oral acute toxicity was considered, the following TEFs were derived: STX = 1, NeoSTX = 1.16, dcSTX = 0.64, GTX1&4 = 1.02, GTX2&3 = 0.60, indicating that the TEFs as defined in the EFSA opinion (2009), particularly in the case of NeoSTX (assumed as toxic as STX in previous studied based on acute i.p. toxicity), require some revision. This is particularly relevant considering the NeoSTX formation by N-oxidation of STX occurring in vivo after ingestion, which can

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be considered a bioactivation reaction. The no acute effect level after oral administration showed this ranking (from the most toxic to the least): NeoSTX 2 fold >STX> dcSTX≈ GTX-1&4 >GTX-2&3. Only one new study on repeated toxicity of NeoSTX is available, showing that only the highest studied dose (6 µg/kg of NeoSTX administered subcutaneously) induced a reduction of weight and food consumption accompanied by a cholestasic pattern. A metabolic pathway was also proposed for the route of metabolization and detoxication of STXs in an *in vitro* study using human liver microsomes, with identification of Vmax and Km, showing for the first time direct evidence for the sequential oxidation and glucuronidation, both being the initial detoxication reactions for the excretion of these toxins in humans.

For PlTX the ARfD is at present derived after sublingual administration, according to the EFSA opinion (2009a): apart from the confirmation of the acute toxicity for PlTX and its analogue 42-OH-PlTX after oral administration and some studies on MoA in vitro and in vivo, it is interesting to consider a short term repeated toxicity study in mice, which can be representative of human exposure scenarios i.e. through repeated consumption of contaminated seafood at least for a short period of time. The study showed that a 7-day-repeated administration of PlTX of doses of 30 mg/kg/day (below the LD_{50}) caused lethality and toxic effects. A NOAEL was estimated at the dose of 3 mg/kg/day, and a LOAEL at the dose of 30 mg/kg/day, with lungs, heart, liver and gastrointestinal tract as the main PlTX targets. However, the NOAEL value can be considered as provisional, due to the limited number of animal used, although it can be a valid basis for further testing. Moreover, some studies showed ultrastructural modifications of skeletal and cardiac muscles, suggesting the cardiac muscle as one of the main PlTX target (in line with signs observed in humans).

Regarding the environmental factors possibly affecting the toxicity of cyanobacteria, most of the studies were again dedicated to MC, and among them > 80% deals with *Microcystis* sp. Both in field and laboratory studies different environmental parameters (nutrient levels, particularly P and N; temperature, CO_2 levels, light, iron, inorganic carbon) were considered with respect to MC production and/or microcystin synthetase (*mcy*) genes expression (the presence of which gives indication about the cell competence in toxin production), but results are quite often controversial. The ELS results and the corresponding inventory evidenced that environment plays a very complex role in the toxicity of the bloom, in terms of fitness of the potentially toxic genotype, of regulation of MC production/quota (e.g. limitation in the quantity of a necessary nutrient, or influence in the regulation of gene expression). It seems clear that elevated/not limiting nutrient levels are the base for an abundant bloom, which is also the assumption for a risky situation, even if the thresholds can be different for the various species. Less clear is the nutrient role in the toxicity of the bloom. Indeed, limiting N can increase the toxicity of the bloom via increased MC production or by shifting the relative ratio of toxic/non-toxic genotype towards higher values, but again some contrasting results are reported.

The number of studies on the other toxins is much less than the one for MCs, and the organisms producing these toxins considered in the studies are more diverse, except for nodularin, produced only by *Nodularia spumigena*, raising more questions than answers.

Regarding mixture toxicity, it should be considered that cyanobacterial blooms are generally characterized by the presence of multiple toxins, or as in the case of PSP toxins they can be inter-converted to other variants once absorbed. Therefore, human exposure to a mixture of cyanotoxins via different routes and media (e.g. drinking water, contaminated food, bathing activity) is very likely. However, the significance of exposure to mixtures of cyanotoxins, potentially via multiple routes and media has not been sufficiently investigated so far. Although interactions can be expected with other contaminants, based on some information on the MoA, very few papers were selected, regarding possible mixture toxicity between cyanotoxins and other chemicals. However, since in many cases health based reference values and the toxicological profile of the single toxins are not sufficiently elucidated, the evaluation of possible mixture toxicity is premature and can be, if any, only qualitative.

Based on the occurrence database, exposure scenarios are tentatively described for the EFSA categories of fish and other seafood, BGAS and crops; consumption of fish/shellfish (especially regarding freshwater organisms) was considered as reported in the EFSA FoodEx, as well as food

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supplement consumption. For the other food items (e.g. crops, game hunting, meat, eggs or dairy products) information was so scant that it was not considered appropriate to describe any exposure scenario. The database on exposure was quite limited, and although some indication was obtained, no definite conclusion on the health risks for the exposed population could be drawn. However, on the basis of the available data, the possibility of risky exposure is evidenced for fish and shell-fish as well for BGAS consumers in relation to MC contamination; for other food items as well as for other cyanotoxins the data base of occurrence is not robust enough to elaborate any scenario. Therefore it is highly recommended to collect more data on both exposure and toxicological profile of toxins. Finally, many data gaps were identified and described.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

This contract/grant was awarded by EFSA to:

Contractor: Istituto Superiore di Sanità

Contract: Review of literature on cyanobacteria toxins in food

Contract number: OC/EFSA/SCER/2014/04

Cyanobacteria are a morphologically diverse group of photosynthetic prokaryotes that occupy a wide range of niches, from freshwater and marine environments to hydrothermal vents, from desert rocks to Antarctic lakes. Several cyanobacteria species produce toxins as secondary metabolites, which can impact on ecosystems, animal and human health (Funari and Testai, 2008; Chorus and Bartram, 1999). On the basis of toxin production, to which humans can be exposed via different routes, the World Health Organization (WHO) has listed cyanobacteria as an emerging health issue, although not considering them as emerging pathogens (Manganelli et al., 2012).

Cyanotoxins are diverse in chemical structure and toxicity. Cyanotoxins fall into three broad groups of chemical structure: cyclic peptides, alkaloids and lipopolysaccharides. Depending on the mechanism of toxicity to invertebrates, cyanotoxins are classified as hepatotoxins (e.g. microcystin, nodularin), neurotoxins (e.g. anatoxin-a, homanatoxin, saxitoxin), cytotoxins (e.g. cylindrospermopsin), dermatotoxins (e.g. lyngbyatoxin), and irritant toxins (e.g. lipopolysaccharides). The most investigated group among cyanotoxins is that of microcystin hepatotoxins (MCs), produced by some strains of several genera of cyanobacteria (*Microcystis, Anabaena, Nostoc and Oscillatoria*). Among more than 100 different structural MCs variants described, the LR congener (MC-LR) is the most studied structural variant and considered the most potent on the basis of its acute toxicity. Tumor promotion by MC-LR has been reported and the International Agency for Research on Cancer (IARC) classified MC-LR as possibly carcinogenic to humans (group 2B) (Cyanobacterial peptide toxins. IARC http://www.iarc.fr/).

Among the several routes by which humans may be exposed to cyanotoxins, the oral route is the most important, occurring by consumption of contaminated drinking water or food. Human exposure from food can be due to consumption of fish, crops, food supplements based on algae, or items of animal origins, following the use of contaminated water for irrigation or in farming activities. A thorough review of scientific data available on the bioaccumulation of cyanobacteria toxins in food has been recently published, evidencing also that studies on MCs are much more numerous than studies on other toxins (Gutiérrez-Praena et al., 2013).

Many different aquatic organisms (fish, bivalves, snails, and other macro-invertebrates) may accumulate cyanotoxins via ingestion of cyanobacteria cells/contaminated food or via the transdermal route as dissolved toxins (e.g. cylindrospermopsin) (Rücker et al., 2007). Cyanotoxins could also be transmitted to plants from surface irrigation waters and toxins can accumulate in edible parts (leaves, roots, fruits, etc.): MCs have been found in several plants destined for animal or human consumption such as ryegrass (*Loliumperenne*), clover (*Trifoliumrepens*), rape (*Brassica napa*) and lettuce (*L. sativa*) (Crush et al., 2008). Some cyanobacteria provide a source of nitrogen to growing rice plants (*Oryza sativa*) where cyanotoxins could accumulate (Rahman et al., 1996). Cyanotoxins uptake can be assumed in animal husbandry through consumption of contaminated waters. Available data seems to indicate negligible residues in meat, milk and dairy products for hydrophilic MC variants, which have low potential for bioaccumulation, but information on lipophilic MC congeners or other cyanotoxins are needed (Orr et al., 2003).

Food supplements containing algae, known as Blue-Green Algae Supplements (BGAS), are mainly derived from *Spirulina* and *Aphanizomenon flos-aquae* (*A. flos aquae*) species. In natural environment (e.g. lakes) they can coexist with other potentially toxic strains of cyanobacteria, such as Microcystis sp. which share the same habitat, causing BGAS product contamination as evidenced in different

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countries (Vichi et al., 2012). In addition, it has been found that *A. flos-aquae* species can produce neurotoxins (Cox et al., 2005).

To protect consumers from the adverse effects of cyanobacterial peptide toxins, the WHO proposed a provisional upper limit in drinking water of 1 µg/L for MC-LR and a Tolerable Daily Intake (TDI) of 0.04 µg/kg bw (WHO 1998 and 2006). The Oregon Health Division set a provisional regulatory standard of 1 µg/g MC-LR equivalents per dry weight product in BGAS (Gilroy et al., 2000). However, this has no legal status outside Oregon, although used for orientation in other countries.

The scientific literature suggests that cyanotoxins can be accumulated in food at concentrations higher than provisional limits set for MC-LR in drinking water. In particular, several investigations on contaminated BGAS have shown levels of contamination exceeding the proposed provisional guidance. Assumptions on the variable daily consumption of these products have evidenced a risk for chronic consumers, independently from exposure to MCs from other possible sources. In addition, as some BGAS are specifically addressed to infants and children with Attention Deficit Hyperactivity Disorders (ADHD), these groups of consumers could be potentially the most exposed to MCs (Dietrich and Hoeger, 2005; Ibelings and Chorus, 2007; Heussner et al., 2012; Vichi et al., 2012).

Data gaps have been identified in the characterization of the toxicological profiles of the different cyanotoxins, and in particular for long term effects in the case of MCs. In the absence of information on the other congeners, on the basis of the highest acute toxicological properties of MC-LR, concentration MC-LR equivalents are usually used as default value for the total concentration of all MC variants. However, the extrapolation from the acute toxicity ranking among MC congeners to the chronic toxicity remains to be demonstrated (Manganelli et al., 2012).

The possibility of changes in the toxicological profiles of the toxins produced by some species, under environmental stress, is an additional issue for potential concern (Bernard et al, 2011). A plausible effect of global warming, on increasing occurrence of cyanobacteria and on physiological and molecular changes in cyanotoxins produced has been evidenced (El-Shehawy et al., 2012). Moreover, aggregate/cumulative exposure has to be considered, covering exposure to the same cyanotoxin via different routes (i.e. drinking water, contaminated food and food supplements) or to different toxins at the same time, and concomitant exposure with other chemicals, such as in the case of organophosphorus pesticides reported to potentiate the anatoxin-induced toxicity (Cook et al., 1988).

1.2. Interpretation of the Terms of Reference

At the kick off meeting (on January 22nd, 2015) some points within the ToR were discussed, clarified and agreed with EFSA.

Limitation of the search strategy:

- Saxitoxins, Palytoxin-group toxins (PlTX) and/or ciguatoxin (CTX)-like toxins: in relation to objective 1 the marine dinoflagellates producing the toxin will be excluded; in relation to objective 2 the key studies used by EFSA in its opinion will be summarized (for data-base completeness); the search will be then limited to paper published after 2009.
- Lipopolysaccharides (LPS toxins): all the Gram-negative bacteria will be excluded
- Aplysiatoxins, Lyngbyatoxins: will be included, although not produced in EU water bodies, due to imported food. However, they will not be the first priority toxins.

Regarding drinking water, it was not directly included in the procurement; however, a range of 'plausible' concentrations will be considered since used for food preparation and included as a possible exposure scenario.

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1.3. Structure of the Report

The methodologies used for the ELS are reported. Then, on the basis of the analysis of the ELS results, data were reported to address:

1) Exposure assessment reporting data on occurrence and analytical methods and the identification of the exposure scenarios

2) Hazard identification and characterisation reporting data on the toxicological profile of cyanotoxins, information on mixture toxicity and on the environmental factors affecting toxicity of a bloom, and the derivation of health-based guidance values (HBGV), when possible.

3) Eventual risk characterisation, by comparing HBGV with exposure drawing conclusions whenever possible.

Data gaps, recommendations as well as any other issue considered relevant for the EFSA risk assessment activities are also reported.

This Final Report is supplemented by 3 Appendices:

1) Appendix A included the information source forms, reporting the queries used in the extensive literature search on the different databases (for the different tasks: occurrence in food, analytical methods, toxicity, environmental factors affecting toxicity and mixture toxicity involving cyanotoxins), the detailed information on the number of initially retrieved papers, the included/excluded papers during the screening phases, and of those papers from which data have been taken and included in the inventory;
2) Appendix B corresponds to the full list of references in an EndNote™ Library, provided as a set of files corresponding to different cyanotoxins; within each file the papers have been further subdivided depending on the matrix in the case of occurrence, and on the species (i.e. fish or mammals) or the toxicity endpoint for the toxicological profile definition; separate EndNote™ files are provided for the environmental factors affecting cyanobacterial toxicity and for the mixture toxicity;
3) Appendix C is the Data Inventory (in tabular format, as a collection of MS Excel spreadsheets, a subset for each cyanotoxin, further subdivided depending on the matrix in the case of occurrence, and in vitro, in vivo and epidemiological studies for toxicity) containing the data extracted from the relevant papers, which can be can be downloaded from the EFSA website in a Microsoft Excel format together with this report.

2. Data and Methodologies

2.1. Methodologies

2.1.1. The protocol for extensive literature search strategy

The literature search strategy and selection criteria (inclusion/exclusion) for the review described in the offer are based on the EFSA Systematic Review Guidance (EFSA Journal 2010; 8(6):1637). It was detailed in Deliverable 1, but when work was in progress some amendments were deemed as necessary; therefore the final strategy (detailed in Deliverable 2) is summarised below:

1. The following literature databases have been consulted: Scopus, Web of Science and PubMed, Agricola, Food Science Source, priority given to information from peer reviewed sources.

2. Searches has been carried out to identify reports, international evaluations or monographs published by agencies such as the Agency for Toxic Substances and Disease Registry (ATSDR), US Environmental Protection Agency (US EPA), World Health Organization (WHO) etc. and national authorities (German BfR, RIVM, Health Canada), by searching in their web site for objective 1. In relation to objective 2 those reports do not describe any original data, but are a sort of review of the

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available original papers and therefore could not be used for data extraction (see also below part regarding inclusion/exclusion criteria).

3. In the original offer as well as in Deliverable 1, in view of an expected low number of retrieved papers, it was foreseen to look at Grey literature, running the search also on Google Scholar and NLM GATEWAY. However, on both those search engines it is not possible to use complex syntax by using parentheses and Boolean connectors, therefore only very simple, generic search could be run, resulting in a large number of records, that could not be processed in an easy way. For example, on Google Scholar the search related to 'Cyanobacteria and toxicity' resulted in 52500 records (much more on Google); 'Microcystin and toxicity' gave 23500 results; the one regarding 'Cylindrospermopsin and toxicity' corresponds to 3160 records, and so on: at a first rapid glance most of them were not relevant, and those being relevant were retrieved by Google Scholar from one of the data-bases we have used (see bullet point 1). Since the number of peer reviewed retrieved papers obtained was quite high, it was decided to give high priority to information from peer reviewed sources and not to consider the results of grey literature, also in view of the fact that 1) the filter could not be changed (i.e. the search term could be present in a citation of another paper, not useful at all for our scope); 2) the screening has to be done one by one, not being possible to export the file in End-Note and check for duplicates and 3) being these environmental contaminants not regulated compounds, no study carried out by any companies according to Guidelines and GLP compliant present in grey literature as 'unpublished report' is expected.

4. ISS and ANSES also consulted some public institutions involved in cyanobacteria and cyanotoxins surveys for data collection.

5. A check of the reference list of the most relevant reviews and publications was also carried out to eventually identify studies which have not been retrieved through other methods.

Search terms were selected to gather information on: 1) occurrence in food and dietary supplements, including bioaccumulation in field and laboratory studies in edible items; toxicokinetic and toxicity data in experimental animals or in in vitro studies, and epidemiology data in humans (hazard identification and characterization); 3) environmental factors responsible for changes in toxicity profiles of cyanobacterial populations; 4) toxicity associated to aggregate/cumulative exposure to different cyanotoxins or concomitant exposure with other chemicals.

For occurrence broad search terms have been initially used (lumping strategy) and then adjusted to the databases being interrogated. Regarding toxicity, although this strategy was initially applied, it turned out not to be very useful (as an example when the term 'cyanobacteria' replaced the term Microcystin* in the search term sequence used in Web of Science, 18512 records were retrieved vs 3253). Good quality papers, to be considered for the definition of the toxicological profile of single toxins, have to indicate which is the cyanotoxin of interest at least once in the title, abstract or text; otherwise the result of the toxicological study cannot be attributed to any of the cyanotoxins relevant for this procurement. Therefore we went to the second phase (referred to as "step 2") in which the name of the toxins was directly used.

All searches have been carried out in English (publications in other languages have been excluded, with the exception of Italian and French, being team members mother tongue), without any 'date of publication' constraint, except for Saxitoxins, Palytoxin-group toxins (PlTX) and/or ciguatoxin (CTX)-like toxins (see section 1.2).

The list of cyanotoxins to be included in the literature search has been agreed by EFSA at the kick-off meeting on January 22nd, 2015.

- Microcystins; MC-LR, MC-YR, MC-RR, MC-AR, MC-LF, MC-LW,MC-FR, MC-deLR, MC-deRR, MC-deLW, MC-deLF, MC-(O)Y, MC-m[Glu(OCH3)]-LR. Since different variants are present, MC names are used in their abbreviated forms, as acronyms, (e.g. microcystins–LR as MC-LR). During the set-up of optimized set of queries we have inserted, connected by the Boolean connector 'OR', as many known acronyms as possible. However, we noticed that 1) in the absence of a general nomenclature rule, each single author can use a different abbreviated form (e.g. microcystins –LR can appear as MC-LR;

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MC-LR; MCYST-LR and so on). In addition, the same acronym can be used to target some other molecule or gene and a number of off topic papers are retrieved. To give an example by using the following acronyms beside Microcystin* [OR "MC-LR" OR "MC-YR" OR "MC-RR" OR "MC-AR" OR "MC-LF" OR "MC-LW" OR "MC-FR" OR "MC-deLR" OR "MC-deRR" OR "MC-deLW" OR "MC-deLF" OR "MC-(O)Y" OR "MC-m[Glu(OCH3)]-LR"] in WoS about 1400 more papers not relevant for cyanotoxins' toxicity are retrieved, and were removed as off-topic after the first screening. With PubMed the situation is even worse, since the search runs checking 'ALL FIELDS' including the Mesh terms (more complex but also more comprehensive): by using the abbreviated forms the number of retrieved records is 45209, since groups of letters such as MC or YR, used to name some genes are recognised. Therefore for the search in Pub Med, the acronyms were not used, since they could invalidate our search with the addition of more than 40000 non-relevant papers. We therefore used only the full name of the toxins, confident to retrieve all the relevant papers, since all the Journals asked to spell out any abbreviation or acronyms at their first appearance in the text, many of them asked for a list of abbreviations, therefore the full name of the toxin appears at least once in the paper.

- Nodularins

- Cylindrospermopsins

- Anatoxin-a, Homoanatoxin-a. When the search term *anatoxin* was used in order to get together all the different analogues and metabolites, many other toxins were retrieved (including Aflatoxin B1 and *Staphylococcus* anatoxin); the 'star' before the term was therefore eliminated and the search terms used were: anatoxin* OR homoanatoxin*. Those toxins other than the cyanobacterial ones were then discarded during the primary screening, since using other strategies we run the risk to loose information and the number of retrieved records allowed to do it.

- Anatoxin-a(S)

- BMAA

- Saxitoxins (carbamoyl-, decarbamoyl-, sulfocarbamoyl- saxitoxins), in this case the names of the marine dinoflagellates producing the toxins will be used as exclusion terms (using the Boolean connector NOT) or alternatively using the Boolean connector AND with the term 'cyanobacteria' for objective 1-occurrence. In relation to objective 1-analytical methods, the name of the marine dinoflagellates will not be used with the Boolean connector NOT as the methods used for the detection/quantification of these toxins of other origin may be relevant. For the toxins already reviewed by EFSA in an opinion, since also the related analytical methods were already reviewed, the search will be then limited to papers published after the opinion. In relation to objective 2 the key studies used by EFSA in its opinion (EFSA, 2009a) will be summarized (for data-base completeness); the search will be then limited to papers published after 2009.

- Lipopolysaccharides (LPS toxins): the term will be associated to the term cyanobacteria by the Boolean connector AND to exclude all the Gram-negative bacteria (also characterised by the presence of LPS, although with some differences). In relation to objective 1-analytical methods, the term 'Gram-negative bacteria' will not be used with the Boolean connector NOT as the methods used for the detection/quantification of these toxins of other origin may be relevant.

- Aplysiatoxins, Lyngbyatoxins: will be included although not produced in EU water bodies due to imported food. However, they will not be the first priority toxins.

- Palytoxin-group toxins (PlTX) and ciguatoxin (CTX)-like toxins: associated to the term cyanobacteria by the Boolean operator AND for objective 1 to exclude production by marine algae regarding the occurrence (objective 1-occurrence). In relation to objective 1-analytical methods, the name of the marine dinoflagellates will not be used with the Boolean connector NOT as the methods used for the detection/quantification of these toxins of other origin may be relevant. For the toxins already reviewed by EFSA in an opinion, since also the related analytical methods were already reviewed, the search will be then limited to papers published after the opinion. In relation to objective 2 the key studies used by EFSA in its opinion (EFSA, 2009b) will be summarized (for data-base completeness); the search will be then limited to papers published after 2009, applying a search

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strategy to select those papers useful for the identification of health based value (acute and repeated toxicological studies). Specifically in the case of ciguatoxins, the most frequently used abbreviation is CTX, which is used as an acronym in several other cases (e.g. to indicate a kind of tumor marker, or a genetic disorder-*Cerebrotendinous Xanthomatosis*- but also the cholera toxin). In these cases in order to restrict the search only to relevant papers, the name of the toxins and its abbreviation were combined with the term CYANOBACTERIA by the Boolean operator "AND".

In addition to the single toxin names, in relation to objective 2 two searches using 1) the term 'cyanobacterial extract' and 2) the generic term cyanotoxin connected by the Boolean connector NOT with the name of specific toxins having their own search were also included (reported in the Source information source as Cyanotoxins 'NOT'). Results are shown below sections.

The searching term sequences (queries) used for the different databases have been reported in the information source form (see Appendix 1), to ensure transparency as well as the possibility for the search to be re-run (reproducibility).

All the records retrieved from the literature search on the different databases have been imported into EndNote™ (see Appendix 2).

2.1.2. The criteria for study selection

Primary screening

The primary screening has been carried out on titles and abstracts, after removal of duplicates by EndNote™. Papers that do not meet the inclusion criteria have been excluded from further analysis.

Inclusion criteria for the task 'occurrence'

- Language (English, Italian and French only).
- Regarding drinking water, it was agreed that although not directly included in the procurement a range of 'plausible' concentrations in drinking water (coming from actual data measured in different EU countries) will be considered since used for food preparation and included as a possible exposure scenario in objective 3.
- Records on zebrafish *in vivo*, medaka *in vivo*, i.p. injection in fish[1], non-edible species of shellfish/fish/gastropods will be included but will be given a low priority for secondary screening.
- Records on exotic animals will be included but will be given a low priority for secondary screening.
- Records on dead animals associated to the quantification of cyanotoxins in the tissues will be included but only when their death is strictly related to a bloom, with the level of cyanotoxins clearly measured.
- Records on macrophytes, aquatic plants, non-edible plants will be included but will be given a low priority.

Exclusion criteria for the task 'occurrence'

Language other than English, Italian and French

- Records on zebrafish embryo, cell fish *in vitro*, fish larvae, ecotoxicity, *Daphnia*, zooplankton, rotifer
- Records on dead animals not related to the quantification of cyanotoxins.
- Records on concentration of cyanotoxins in fresh algal bloom or mats.

Inclusion/Exclusion criteria for the task 'analytical methods'

[1] Records on ip injection in fish will be considered in objective 2 (Search 1&2 toxicokinetic/toxicity).

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- Language (English, Italian and French only).
- All matrices included

Inclusion/Exclusion criteria for the task 'international/national regulations/guidelines or "health alert" levels'

- Language (English, Italian and French only).
- Excluded: records on the physiological regulation of toxin production by cyanobacteria.
- Excluded: records on recreational/bathing water monitoring, water treatment.

Inclusion/Exclusion criteria for the task 'hazard identification and characterization'

- Language (English, Italian and French only)
- Excluded: records on plants and invertebrates.
- Excluded: records on occurrence (even when measured as inhibition of enzyme activity or Mouse bioassay).
- Excluded: records related to the use of cyanotoxins as probes or inhibitors (e.g. of phosphatase in the case of microcystins).
- Excluded: records regarding toxins other than cyanotoxins.

The number of publications excluded from further analysis has been recorded in the information source form (See Appendix A), but the papers were deleted from the EndNote™ files. Whenever there was uncertainty about the relevance of the paper on the basis of the only title and abstract, it was considered to be better evaluated by analyzing the full text.

All titles and abstracts retrieved from the literature search and meeting the primary screening criteria have been placed in a dedicated group in EndNote™ (Appendix B). Data have been divided into subset for each group of cyanotoxins (e.g. microcystins, nodularins, etc.).

Secondary screening

The full text of each article that passed the primary screening has been retrieved for secondary screening electronically or, when not possible, as paper copy. Only in few cases, e.g. some book chapter or old papers, the retrieval was not possible, but this was not considered as a problem, since book chapters are not usually dealing with original data (see below on secondary screening exclusion criteria) and dated information do not generally meet the present quality standard and are not used for risk assessment.

During the secondary screening, a more in depth look at the content allowed to further exclude some papers, for which the actual meeting of the primary screening exclusion criteria was uncertain by only reading the title and abstract (e.g. text in Chinese, uncharacterized crude cyanobacterial extracts).

Aspects such as the design, execution, methods for analysis and reporting of the study, which may lead to give a biased result has been considered on the full text. The Klimish score is used to assess the reliability of toxicological studies: those having a score=4 (K4) were excluded. That scoring system may not be relevant for papers on analytical methods, occurrence data or on the environmental factors affecting toxicity. In such situations, other quality criteria have been considered and described below.

For the search regarding the effect of environmental factors on cyanobacteria toxicity, a similar score system was used from 1-4, based on the following parameters:

- appropriateness of the approach or experimental design;
- appropriateness of the statistical analyses;
- adequacy of experimental techniques;
- soundness of conclusions and interpretation.

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When all the parameters were satisfied, a score of 1 was assigned (meaning good quality ones); any weak aspect justifying score 2 and 3 were annotated in the note field of the inventory. Papers with a score =4 were excluded.

Inclusion/exclusion criteria for the task 'occurrence'

- Only records providing a description of the analytical method used and sufficient information on the number of samples and the species will be included in the inventory.
- Records with insufficient information on the study design (e.g. animal genera, number of samples, the analytical method used) will be excluded.

Considering the large number of records retrieved on occurrence of MCs in food of animal origin, a priority score was attributed based on the title and abstract according to the expected relevance.

- High priority when the results of MCs in edible tissues are present in the abstract.
- Medium priority when there are no results of MCs in the abstract and it is not sure to find them in the full article.
- Low Priority when there are no results of MCs in the abstract and it is not expected to find them in the full paper (the study doesn't seem to include this parameter).

All the records given a high and medium priority score have been screened for possible inclusion in the inventory and their data included when they met the inclusion criteria.

The records given a low priority score have not been screened but are included in the EndNote library for possible further consideration.

These priority criteria have not been applied to the other groups of cyanotoxins. All the eligible records have been reviewed for possible inclusion in the inventory.

For papers on occurrence of MCs in crops the score was:

- High priority to the papers with results in the abstract or indication that they should be present in the full paper.
- Low Priority to the papers for which it is not expected to find results of MCs in the tissues in the full papers (because the study doesn't seem to include this parameter).

All the records given a high priority score have been screened for possible inclusion in the inventory.

All the records retrieved on occurrence of MCs in food supplements passing these criteria were screened for inclusion in the inventory.

Inclusion/exclusion criteria for the task 'analytical methods'

- Due to the important number or records, priority has been given to those with performance criteria and/or comparing different techniques.
- Priority has been given to the records dealing with cyanotoxins but those dealing with LPS, and also toxins of other origin (e.g. marine dinoflagellates) have been considered.

Inclusion/Exclusion/Priority criteria for the task hazard identification and characterization'

- Excluded: records with insufficient information on the number of animals, description of methodology used, or very poor reporting (Klimish score 4; environmental factors score 4),or related to environmental factors affecting parameters other than toxicity
- Excluded: records related to extracts were considered only when compared in the same paper with the same amount of pure toxins. They were parked in a specific folder: this approach allows the possibility to use them in case EFSA could be interested in understanding the toxicity of crude extracts.

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- Excluded: Records with insufficient information on the toxins used as test item and its quantification.
- Excluded: records corresponding to reviews and book chapters (on cyanotoxins as a general topic or on risk assessment related issues); they were grouped and not used for data extraction, since they do not report original data. Otherwise the same result could be present many times (coming from different reviews), altering the possible future consideration of the Weight of Evidence approach, without the possibility to check in some other cases for the quality of the original data.
- Records related to *in vivo* acute/repeated/specific target toxicity studies on mammals from which it is possible to derive a reference value were given first priority for data extraction. ISS noted that in many cases toxicity studies have been carried out with a single dose: it was agreed with EFSA that when other well conducted multiple dose toxicity studies are available, the single dose studies will be excluded from data extraction, being not useful for any reference value derivation. However, in case of no other study is available they were included on the basis of an expert judgment approach.
- It was agreed with EFSA that toxicity data on fish or other species have to be parked in a specific folder and not further processed for data extraction. The same for case studies on poisoning episodes with wildlife or domestic animals (dogs, sheep, cow, horses).This approach allows the possibility to use them if deemed necessary, in a possible future follow up activity.
- Records related to epidemiological studies were used for data extraction, providing they meet quality criteria, but case-studies on human poisoning were parked in a specific folder and not further processed for data extraction.
- Records related to papers dealing with *in vitro* data (except for genotoxicity studies which were included as a first priority also when carried out *in vitro*) were parked in a specific folder and not further processed for data extraction, since it is at the moment not possible to obtain quantitative information useful for the derivation of a reference value from this kind of studies.
- Records related to papers dealing with MeA/MoA of toxin-induced toxicity (qualitative toxicodynamic data) were parked in a specific folder and not further processed for data extraction, since it is at the moment not possible to obtain quantitative information useful for the derivation of a reference value from this kind of studies.

From bullet points 5 to 9 criteria have been modified with respect to what is included in the draft report on the basis of discussion with EFSA. These additional exclusion criteria and prioritization were considered necessary due to the large number of papers eligible for data extraction (e.g. 618 for MCs in relation to hazard identification and characterization), most of which were considered of limited value for the derivation of a reference value.

All the studies belonging to the low priority folders have been further divided into subcategories in order to made explicit the kind of available study: e.g. in the case of MoA a subdivision considering the molecular, cellular, organ, organism levels of organization of the experimental models and techniques will be indicated.

2.1.3. Data extraction and design of the inventory

Data from eligible study after the secondary screening has been extracted and captured in a data extraction form, representing the inventory of the easy-to-use-excel database. With respect to the inventory originally agreed and described in Deliverable 1, some substantial improvements have been introduced, following specific requests from EFSA for both occurrence and toxicity data.

Regarding the inventory on Occurrence data, it was agreed to add a column reporting a score of reliability for the used analytical methods. The score is as follows:

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1 = reliable without restriction (validated method),
2 = reliable (fully characterized method),
2 = reliable with restriction (only recovery included),
3 = not reliable (no recovery included).

Regarding toxicity data, as it was agreed with EFSA, when authors describe results within the text as data not shown, it was decided not to consider them, unless they refer to fully negative results in comparison with positive results of sufficient quality shown in the paper. When data were only graphically reported (e.g. histograms in Figures), only qualitative data (e.g. induction of apoptosis) were reported. When relevant, a snapshot of the picture was included in a column 'Attachment' included in the inventory. Analogously when data were reported in very complex tables (e.g. many haematological parameters obtained at different times and doses of treatment), beside the description as a text (up to 250 characters, as indicated in the general EFSA format) a snap-shot of the table was included.

Regarding the effect of environmental factors on cyanobacteria toxicity, the inventory was improved on the basis of data found in the retrieved papers, and was created as a new format, since parameters to be reported are pretty different from the one typical of toxicological studies.

A field 'Comments' is included, in which any relevant issue was annotated.

Data have been divided into subset for each group of cyanotoxins (e.g. microcystins, nodularins, etc.). Some fields have fixed choice (e.g. for quality criteria the score is compulsory) to increase reporting harmonization/standardization.

2.1.4. Quality check

Although the amount of records retrieved was large, about 5% of the papers (randomly selected) screened by each operator has been separately re-screened by a senior scientist. No striking disagreement occurred, so that it was not necessary to involve a 3rd expert. In addition, whenever a doubt was present in one of the operators, it was immediately discussed with a senior scientist, possibly with all the people involved in the screening so that the harmonization of the expert judgment was maximized. With this procedure more than 10% of the papers were actually cross checked.

The same approach has been followed for the quality check of data extraction.

3. Results

The number of papers retrieved for each search was much higher than expected; many of them resulted to be not relevant and therefore excluded, but a considerable amount of paper were analysed and found eligible for data extraction. The detailed description of the number retrieved from the initial search, duplicates removed, exclusions due to the primary and secondary criteria, 'priority' scoring for data extraction (for objective 1-occurrence and objective 2-toxicological profile, in this latter case with further subdivision in different categories) are reported in detail in the information source form (see Appendix A) and discussed in the interim Reports (Deliverables 2 and 3) and will not be repeated here. Below a narrative description of the obtained results is included.

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3.1. Occurrence

3.1.1. Microcystins

Data coming from the ELS were divided in 3 subsets depending on the food category: 1) food of animal origin; 2) food supplements; and 3) edible crops. Individual data in the data inventory are available in Appendix C in tabular format, as MS Excel spreadsheets, which can be downloaded from the EFSA website in a Microsoft Excel format together with this report

1) Food of animal origin

a) aquatic edible species

The highest worldwide concentrations of free microcystins (expressed as MC-LR eq) reported in **fish** muscle from field studies are:

- 3830 µg/kg FW in silver carp (*Hypophthalmichthys molitrix*) from Brazil (Oliveira et al. 2013) by HPLC-MS;
- 2860 µg/kg FW in silver carp (*Hypophthalmichthys molitrix*) from China (Zhang et al. 2007) by LC-MS;
- 1917 µg/kg FW in a ray-finned fish (*Haplochromis spp*) from Uganda (Poste et al. 2011) by ELISA;
- 500 µg/kg in a bream (*Abramis brama*) by HPLC-DAD and 350 µg/kg FW in roach (*Rutilus rutilus*) by GC-MS from Poland (Pawlik-Skowrońska et al. 2012, 2013);
- 340 µg/g FW in Argentinian silverside (*Odontesthes bonariensis*) from Argentina (Cazenave et al. 2005 erratum 2006) by LC-MS;
- 337 µg/kg FW in tilapia (*T. rendalli*) from Brazil (Magalhaes et al. 2001) by ELISA;
- 119 µg/kg FW in common carp (*Cyprinus carpio*) from Greece (Papadimitriou et al. 2012b) by ELISA.

High concentrations were also reported in muscles of **frogs** (*Rana epirotica*) with 325 µg/kg FW, crayfish (*Astacus astacus*) with 329 µg/kg and whole body of freshwater **shrimps** (*Atyaphyra desmaresti*) with 2250 µg/kg FW (Papadimitriou et al. 2012b) by ELISA.

According to a review of Vasconcelos (1999) the highest reported MCs concentrations (MC-LR) in edible parts were 2700 µg/kg in crayfish and 16000 µg/kg in **mussels** (original reference of the studies not mentioned).

Saltwater mussels (*Mytilus edulis*) from Canadareached 3400 up to 63400 µg/kg FW by GC-MS with Lemieux oxidation for total free + bound MCs. By phosphatase inhibition, concentrations of the same samples gave a range of 15 to 22 µg/kg FW for free MCs (Williams et al., 1997b).

Regarding **European countries**, the range of reported concentrations of MCs in fish muscle from field studies is as follows:

- Czech Republic: below the limit of detection (0.13 µg/kg FW) in common carp (*Cyprinus carpio),* pikeperch *(Sander lucioperca),* perch (*Perca fluviatilis),* pike (*Esox Lucius),* asp (*Aspius* aspius), European eel *(Anguilla anguilla)* and grass carp (*Ctenopharyngodon idella)* by HPLC-MS (Kopp et al., 2009, 2013).
- Greece: 119 ± 33 and 95 ± 31 µg MC-LR eq/kg FW in common carp (*Cyprinus carpio)* (Papadimitriou et al., 2012b) by ELISA; 30 µg MC-LR eq/kg FW in common carp (Papadimitriou et al., 2013) by ELISA; 19 ± 2.6 µg MC-LR eq/kg FW in Acheloos roach (*Rutilus panosi*) (Papadimitriou et al., 2012a) by ELISA; 16 µg MC-LR eq/kg FW in Prussian

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carp (*Carassius gibelio*) (Kagalou et al., 2008) by ELISA; 7.1 µg MC-LR eq/kg FW in Prussian carp (Papadimitriou et al., 2010) by ELISA; up to 1499 µg MC-LR eq /kg DW in Danube sturgeon (*Acipenser gueldenstaedtii*) by ELISA (Gkelis et al., 2006), however HPLC analysis did not reveal the presence of MCs.

- Italy: from 0.45 to 36.42 µg MC-LR eq/kg FW in flathead grey mullet (*Mugil cephalus*) by ELISA (Bruno et al., 2009).
- Poland: from below the limit of detection to 500 µg/kg FW in bream (*Abramis brama*) by HPLC-DAD (Pawlik-Skowrońska et al. 2013), 350 µg/kg FW in roach (*Rutilus rutilius*) and 217 µg/kg FW in perch (*Perca fluviatilis*) by GC-MS (Pawlik-Skowrońska et al. 2012).
- Spain: from 1.5 to 22.7 µg/kg DW in tench (*Tinca tinca*) by ELISA (Moreno et al., 2011); 5.2 ± 0.6 µg MC-LR eq/kg FW in mugilid (*Liza sp*) by ELISA (Romo et al., 2012); below the limit of detection by LC-MS in tench (Rios et al., 2013).

In saltwater mussels:

- Greece: from 45 ± 2 to 142 ± 14 µg MC-LR eq/kg FW (Vareli et al., 2012) by ELISA.

In crayfish:

- Greece: 329 ± 95 and 216 ± 66 µg MC-LR eq /kg FW (Papadimitriou et al., 2012b) by ELISA; from 84 to 230 µg MC-LR eq/kg DW (Gkelis et al., 2006) by ELISA.
- Italy: 45.45 µg MC-LR eq /kg FW by ELISA (Bruno et al., 2009).
- Spain: mean of 49 ± 39 µg MC-LR+RR eq/kg DW up to 90 ± 28 µg MC-LR eq/kg DW (Rios et al., 2013) by LC-MS.

In frogs:

- Greece: 88 ± 21 and 325 ± 87 µg MC-LR eq/kg FW (Papadimitriou et al., 2012b) by ELISA.

When considering the reliability of the analytical method, only few data remain from field studies (expressed as fresh weight, **in bold data for European countries**):

<u>In fish muscle</u>:

- 2860 µg MCs/kg FW in silver carp (*Hypophthalmichthys molitrix*) from China for the sum of MC-RR and LR by HPLC-MS (Zhang et al. 2007);

- 340 µg MC-RR/kg FW (max) and 130 ± 180 µg MC-RR/kg FW (mean ± SD) in Argentinian silverside (*Odontesthes bonariensis*) from Argentina by LC-MS (Cazenave et al. 2005 erratum 2006);

- 119 ± 33 µg MC-LR eq/kg FW (mean ± SD) in common carp (*Cyprinus carpio*) from Greece (Papadimitriou et al. 2012b) by ELISA; 29.83 µg MC-LR eq/kg FW (mean) in (Papadimitriou et al. 2013); 3.5 µg MC-LR/kg FW (max) from USA by LC-MS/MS (Schmidt et al. 2013);

- 70 µg MC-LR µg/kg FW (max) in black crappie (*Pomoxis nigromaculatus*) from USA by LC-MS/MS (Schmidt et al. 2013);

- 19.03 ± 2.56 µg MC-LR eq/kg FW (mean ± SD) in Archeloos roach (*Rutilus panosi*) from Greece by ELISA(Papadimitriou et al., 2012a);

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- 16.13 µg MCs/kg FW in silver carp (*Hypophthalmichthys molitrix*) from China as the mean for the sum of MCs (MC-LR, -RR, -YR) by LC-MS (Chen et al., 2006);

- 16.05 ± 11.97 µg MC-LR eq/kg FW (mean ± SD) in Prussian carp (*Carassius gibelio*) from Greece by ELISA (Kagalou et al., 2008) and 7.1 ± 2.5 µg MC-LR eq/kg FW (Papadimitriou et al., 2010);

- 3.9 ± 2.2 µg MCs/kg FW in Argentinian silverside (*Odontesthes bonariensis*) from Argentina as the mean ± SD for the sum of MCs (MC-LR,-RR,-YR,-LA) by HPLC-MS/MS (Ame et al., 2010);

- below detection limit (70 MCs µg/kg FW) in Tilapia, catfish, trout, salmon, arctic char from Canada for the sum of MCs (MC-LR,-RR,-YR,-LA) by LC-UV (Niedzwiadek et al. 2012);

- below detection limit (0.24 MC-LR µg/kg FW) in bluegill (*Lepomis macrochirus*), channel catfish (*Ictalurus punctatus*), largemouth bass (*Micropterus salmoides*) from USA by LC-MS/MS (Schmidt et al., 2013).

In bivalves:

- 3400 ± 1000 µg total Mc-LR eq/kg FW (mean ± SD) by GC-MS with Lemieux oxidation in blue mussels (*Mytilus edulis*) from Canada but below detection limit (not given) in blue mussels from USA (Williams et al., 1997b);

- 141.5 µg MC-LR eq/kg FW (max) in Mediterranean mussel (*Mytilus galloprovincialis*) from Greece by ELISA for whole tissues (Vareli et al., 2012).

In crustaceans:

- 329 ± 95 µg MC-LR eq /kg FW (mean ± SD) in crayfish from Greece by ELISA (Papadimitriou et al., 2012b);

- 21.3 ± 37.5 µg MC-LR eq/kg FW (mean) and 105 µg MC-LR eq/kg FW (max) in blue crab (*Callinectes sapidus*) from USA by ELISA (Garcia et al., 2010).

In frogs:

- 325 ± 87 µg MC-LR eq/kg FW in frogs from Greece by ELISA (Papadimitriou et al., 2012b).

Cooking (boiling in water, microwave) may result in an increase of the MCs concentration by 28%-36% (Bruno et al., 2009), 57%-213% (Freitas et al., 2014) up to 300% (Zhang et al., 2010). However, other authors reported no effect (Morais et al., 2008) or even a decrease (Guzman-Guillen et al., 2011).

The data collected show the broad occurrence of microcystins in measurable concentrations in **aquatic edible species** from European and non-European countries (North America, South America, African countries, Asian countries) confirming that accumulation of microcystins is a global concern.

In the liver, the concentrations are 10 to 20 times higher than the concentrations in the muscle in some studies (Xie et al., 2004, Adamovský et al., 2007, Kozlowsky-Suzuki et al., 2012, Pawlik-Skowrońska et al., 2012) but this relationship is not observed in other studies (Shen et al., 2005, Xie et al., 2005, Zhao et al., 2006a-b, Chen et al., 2007b, Zhang et al., 2007, 2009a, Wilson et al., 2008).

No evidence for biomagnification was found (Ibelings et al., 2005, Papadimitriou et al., 2012b).

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According to Kopp et al (2013) there is no clear and direct relation between the fish species with different feeding strategies and MCs concentrations measured in their tissues.

b) Livestock

The few results available on livestock rely on a very limited number of animals (2 or 4 depending on the study); further research is needed before drawing a conclusion regarding the possible transfer of MCs to the milk or the meat (with a more accurate analytical method and taking into account the bound-MCs).

One study reported a concentration up to 920 µg/kg FW in the liver of beef cattle by ELISA exposed 28 days to cells *Microcystis aeruginosa* in drinking water (Orr et al., 2003). However, HPLC and GC-MS analysis detected no MCs in the samples. The analytical method is considered not reliable.

The study of Manubolu et al. (2014a) has to be highlighted as it shows the presence of MCs in the blood of cows grazing on a pasture along Baltic Sea, with a concentration up to **0.125 µg MC-LR eq/L blood** by ELISA.

c) Birds and game hunting

Only one study measured MCs in the muscle of wild birds. The mean concentration (MC-RR, -YR, -LR) was 15.17 µg/kg DW in duck (*Anas platyrhynchos*) and 3.026 µg/kg DW in water bird (*Nycticorax nycticorax*) from China by LC-MS (Chen et al., 2009b).The analytical method is considered reliable.

Only one study reported the concentration of MCs in the liver of a wild roe deer (*Capreolus capreolus*) found almost dead in Norway. The concentration was 1361 µg/kg FW for the sum of MC-YR, -LR and –RR (1255 ng MC-YR/g, 68 ng MC-LR/g and 38 ng MC-RR/g) by LC-MS (Handeland and Østensvik (2010). The analytical method is considered not reliable.

2) Food supplements

Regarding European countries, the range of reported concentrations of MCs in food supplements is as follows:

- Germany: from not detected (10 µg/kg DW) to 5800 µg MC-LR eq/kg DW in *A. flos-aquae* products by LC-MS/MS and 6100 by adda-ELISA. No MCs were detected in *Spirulina* and *Chlorella* products by LC-MS (Heussner et al., 2012).
- Ireland: from not detected to 2210 µg MC-LR/kg in *A. flos-aquae* products harvested from Klamath Lake (USA) by LC-MS/MS (Vinogradova et al., 2011).
- Italy: from not detected to 5200 µg/kg, with 40% of samples showing levels above 1000 µg/kg (Vichi et al., 2012) in *A. flos-aquae* products by LC-MS/MS for 7 congeners of MCs (only MC-LR and MC-LA detected); below the limit of detection in *Spirulina* products. From 900 to 4600 µg MC-RR+LA/kg in pills, from 140 to 1420 µg/kg for MC-LR, -YR, -LA, -RR and two demethylated forms of MC-RR in capsules (Bruno et al., 2006).
- Internet distributors: from 20 to 380 µg MC-LR eq/kg by ELISA (Saker et al., 2005).

Regarding non-European countries:

- In Canada: from 500 to 35 000 µg/kg by LC-MS (Lawrence et al., 2001).
- In China: from not detected to 163 µg/kg (mean = 14 ± 27 µg/kg) in *Spirulina* products by LC-MS/MS for MC-LR, -RR, -YR (Jiang et al., 2008).
- In Switzerland: from not detected to 4000 µg MC-LR+LA/kg in *A. flos-aquae* products from Klamath lake (USA) by UPLC-MS. No MCs were detected in *Spirulina* products (Ortelli et al., 2008).

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- In Thailand: from not detected to 78 µg MC-LR eq /kg in products with Spirulina, blue-green algae, or Chlorella as major components (Yu et al., 2002) by ELISA.
- In USA: mean ranged from 2150 to 10890 µg MC-LR eq/kg in *A. flos-aquae* products according to the brand (4 brand tested) and the year (1996 to 1999) by ELISA. The maximal concentration was 18400 µg MC-LR eq/kg. In *Spirulina* products, the mean ranged from 150 to 0520 µg MC-LR eq/kg. The maximal concentration was 2120 µg MC-LR eq/kg (Gilroy et al., 2000).

When considering the reliability of the analytical method, few data remain (expressed as dry weight, **in bold data for European countries).**

In *A. flos-aquae* products:

- mean ranged from 2.15 to 10.89 µg/g in products from USA by ELISA (Gilroy et al., 2000);

- 0.86 µg MCs/g (mean) and 5.20 µg MCs/g (max) in products from Italy for the sum of MCs (MC-RR,-YR,-LR,-LA) by LC-MS/MS (Vichi et al., 2012);

- 4 µg/g (max) for the sum of MCs (MC-RR,-YR,-LR,-LA) in products from Switzerland by UPLC/MS (Ortelli et al., 2008);

- 2.531 µg MC-LR eq/g (max) in products from Ireland by surface plasmon resonance (SPR) immunobiosensor assay (Vinogradova et al., 2011);

- 0.050 µg MC-LR eq/g (mean) in products from Taiwan by ELISA (Yu et al., 2002);

In spirulina products:

- mean between 0.15 and 0.52 µg/g in products from USA by ELISA (Gilroy et al., 2000);

- 0.014 µg MCs/g (mean) and 0.163 µg MCs/g (max) in products from China for the sum of MCs (MC-LR,-RR,-YR) by LC-MS/MS (Jiang at al., 2008);

- 0.047 µg MC-LR eq/g (mean) in products from Taiwan by ELISA (Yu et al., 2002);

- below detection limit (0.0012 to 0.015 µg/g according to the variant) in products from Italy for the sum of MCs (MC-RR,-YR,-LR,-LA) by LC-MS/MS (Vichi et al., 2012);

- below detection limit (0.1 µg/g) in products from Switzerland for the sum of MCs (MC-RR,-YR,-LR,-LA) by UPLC/MS (Ortelli et al., 2008);

In *Chlorella* products:

- from below detection limit (0.01 µg/g) to 0.036 µg MC-LR eq/g in products from Taiwan by ELISA (Yu et al., 2002).

3) Edible crops

Regarding **European countries**, only one study reported concentrations of MCs in edible crops in field conditions:

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- in England: MCs in lettuce grown for sale and irrigated with contaminated water ranged from 94 to 2487 µg/kg DW by colorimetric immuno-protein phosphatase inhibition assay (CIPPIA) (Codd et al., 1999).

Regarding non-European countries:

- In China: MCs in rice grain grown in natural conditions ranged from 0.04 to 3.19 µg MC-LR eq/kg DW by ELISA (Chen et al., 2012a); in water chestnuts (*Trapa natans*, one of the most popular vegetables in Asian countries) the highest level of MCs was 1.68 µg/kg FW by ELISA confirmed by LC-MS (Xiao et al., 2009).
- In Guatemala: 1.16 µg/kg DW in wolf apple (*Solanum lycopersicum*) and 1.03 µg/kg DW in peppers (*Capsicum annuum*) irrigated with water contaminated with MCs, by HPLC (for the sum of MC-LR, MC-RR, MC-YR) (Romero-Olivia et al., 2014).
- In Saudi Arabia: 1200 µg/kg FW in radish and 70 µg/kg FW in cabbage by ELISA collected from farms using contaminated groundwater for irrigation (Mohamed et al. (2009).

When considering the reliability of the analytical method, only one study remains from field studies regarding water chestnut from China but it is not sure if the results are expressed as fresh weight or dry weight (lyophilized samples). **No data remain for European countries.**

3.1.2. Cyanotoxins other than Microcystins

Data coming from the ELS were divided in 3 subsets depending on the food category: 1) food of animal origin; 2) food supplements; and 3) edible crops. Individual data in the data inventory are available in Appendix C in tabular format, as MS Excel spreadsheets, which can be downloaded from the EFSA website in a Microsoft Excel format together with this report

Some publications have used several analytical methods for concentration measurement. In that case, the different values are indicated, each analytical method distinguished with a different color.

Generally, the confirmation of the toxin was done using LC/MS but the quantification has apparently been performed using HPLC with different detections

The distribution of the papers and the data recorded depending on the type of toxins considered are shown in table 3.1.2.1

The toxins nodularins, BMAA, cylindrospermopsins and anatoxins have been the most investigated. Numerous data come from laboratory studies where accumulation in different types of organisms has been tested. Only half of the publications related to their occurrence in animals through field studies but in some cases data concerning the toxin production by cyanobacterial blooms are not available.

The PSP toxins have also been investigated but more rarely.

Data concerning lyngbyatoxin and its congeners are scant: only 6 studies have been referred to but none could be regarded as occurrence in food.

If some publications refer to some CTX-like toxins probably produced by cyanobacteria in some ciguateric regions, the results did not enable to identify the toxin and consequently, no quantification could be established.

No publication dealing with palytoxin-like toxins was found in the inventory. Some data may be available but they will be linked to microalgae and not cyanobacteria.

Concerning lyngbyatoxins and palytoxins, some data are expected to be found in old references for which the whole publication was not available. Anyway, those data would be very few and the method for detection might be not well described and not responding to the present standard.

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Some studies have been conducted on food supplements derived from blue green algae (from *Spirulina* and *Aphanizomenon*), at least for anatoxins and cylindrospermopsin. They show some variability in their results with detection of toxins in few cases.

Table 3.1.2.1: Distribution of papers and data depending on the type of toxins

Toxin	Occurrence in	Water	Fish	Shellfish	Food supplements	Others	N° of papers
Anatoxins	Food	1	5	3	3	0	11
	Dead animals	3	0	0	0	7	7
	Non edible matrix	1	0	0	0	1	1
	Review	0	1	0	0	1	1
	Excluded	6	0	0	0	1	6
Total							26
CYN	Food	1	14	2	1	7	21
	Dead animals	0	0	0	0	0	0
	Non edible matrix	0	0	2	0	1	3
	Review	0	4	3	1	3	4
	Excluded	0	0	0	0	0	0
Total							28
Lyngbyatoxin	Food	0	0	0	0	1	1
	Dead animals	0	0	0	0	2	2
	Non edible matrix	2	0	0	0	2	2
	Review	0	0	0	0	0	0
	Excluded	1	0	0	0	0	1
Total							6
Nodularins	Food	5	15	11	0	14	29
	Dead animals	0	0	0	0	1	1
	Non edible matrix	0	0	0	0	1	1
	Review	0	3	2	0	2	3
	Excluded	3	0	0	0	0	3
Total							37
BMAA	Food	5	10	8	0	17	24
	Dead animals	0	0	0	0	1	1
	Non edible matrix	0	0	0	0	3	3
	Review	1	1	0	0	2	2
	Excluded	0	0	0	0	0	0
Total							30
PSP toxins	Food	5	5	3	0	1	8
	Dead animals	1	0	0	0	1	1
	Non edible matrix	0	0	0	0	0	1
	Review	0	1	1	0	1	1
	Excluded	2	1	0	0	0	3
Total							14

When the data on toxin concentration from cyanobacteria (whether culture or bloom samples) are presented, it is frequently given the intracellular concentrations but the soluble part in the water column or the medium is not commonly quantified.

It was also not clear to distinguish when the values reported take into account the % of recovery. In some cases, this % can be very low. A key issue is that toxin levels may differ largely from one method of detection to the other (such as LC/MS compare to ELISA) as shown by Karlsson et al 2003.

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Below, the data remained after the further check for the quality of analytical data are schematically reported with the actual levels founds, subdivided toxin by toxins. **Data referring to European Countries are in bold.**

ATXs

1) Food of animal origin

Only 3 publications deal with the presence of ATXs in **fish** with reliable analytical methods, **none in Europe**. In two of them (Al-Sammak et al 2014 and Galvao et al 2009), no ATX was detected by HPLC-FD in different fish species (carp, bass, white crappie, catfish, shad, walleye, wiper, bluegill) from US Nebraska locations as well as from Brazil in Tilapia, respectively. These two publications rely on fully characterized analytical methods. The publication by Pawlik-Skowronska et al 2012 also showed some accumulation of ANTX a by HPLC-FD in fish muscle of different species (*Carrasius gibelio*, *Rutilus rutilus* and *Perca fluviatilis*) but the unit of the values was inconsistent between the graph and the text. Only one paper (Al-Sammak et al 2014) investigated the portion of free and bound toxin.

In shellfish, only two field studies have investigated the accumulation of ATX-a and homo-ATX a in giant clams (*Tridacna maxima*) from New Caledonia (Laurent et al 2008 and Mejean et al 2010). However, if suspected in these samples, the toxins were not quantified and the method used was no reliable.

2) Food supplements

In **European countries, 4 publications deal with the presence of ATXs in food supplements**:

- Heussner et al 2012 did not detect ATX a in 18 samples of *A. flos-aquae, Spirulina* and *Chlorella* products from the German market by LC-MS/MS
- Similarly, Rellan et al 2009 did not detect ATX in *Spirulina* or *Aphanizomenon flos-aquae* food supplements using LC–FL followed by GC–MS
- Rawn et al 2007 also did not detect ATX a in 4 food supplements samples (3 BGAS and 1 *Spirulina*) sold in Canada using LC-MS/MS
- The publication of Draisci et al 2001 is the only one reporting *Spirulina* tablet and capsule samples of different brands collected from commercial suppliers in Rome (Italy) with 10000 µg/kg of dihydrohomo-anatoxin a in 3 out of 5 samples and of epoxyanatoxin-a in 2 out of 5 samples.

3) Edible crops

No data available.

NODULARINS

1) Food of animal origin

In fish, these values are reported (**none in European Countries**):

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- In Australia, Stewart et al 2012 showed a level of NOD up to 17.6 µg/kg fw in sea mullet (*Mugil cephalus*) by LC/MS-MS while no CYN was detected in other species (greasy rockcod, longfin eel, yellowfin bream and some unidentified mullets);
- Van Buynder et al 2001 concluded that the level of NOD was below 150 µg/kg in the flesh of tailor, flounder, garfish and snapper but reached 2.5 µg/kg in the flesh of finfish from lake Victoria by LC/MS-MS;
- From Brazil, no NOD was detected in tilapia fish muscle by LC/MS (Galvao et al 2009);
- From the gulf of Gdansk (Poland) who reported a maximum of 347.3 ± 564.2 µg/kg dw in the muscle of fish (*Neogobius melanostomus*) by HPLC (Marzur-Marzec et al 2013).

Several publications deal with NOD levels in shellfish but for most (Barda et al 2015, Chen et al 1993, Karlsson et al 2003, Marzur-Marzec et al 2007, Persson et al 2009, Sipia et al 2012) the analytical method descriptions do not allow considering these data for risk assessment. Only three publications can be retained:

- Van Buynder et al 2001 who obtained a maximal level of 2500 µg/kg in mussel by HPLC-MS/MS
- Sipia et al 2008 who reported a maximum of 79,5 µg/kg dw in soft tissue of mussel (*Mytilus edulis*) from the Baltic Sea (Finland) by LC/MS
- Marzur-Marzec et al 2013 who reported a maximum of **816.5 µg/kg dw in mussel (*Mytilus trossulus*) from the gulf of Gdansk (Poland)** by HPLC.

In Australia, Van Buynder et al 2001 investigated the effect of cooking on prawn containing NOD and they showed by HPLC-MS/MS an increase of NOD concentration from 10.3 to 46 µg/kg respectively, due to the redistribution of the toxin between the viscera and the flesh. The same authors also showed that the highest level of NOD in mussel and prawn was detected during the bloom of *Nodularia spumigena* and that this level decreased few days after the bloom.
The proportion of the bound toxin compared to the free one has not been investigated.

Few publications have investigated the level of NOD in crustacean (**none in Europe**):

- In New Zealand, Wood et al 2012 reported a maximum of 0.7 µg/kg fw NOD in the tail tissue of freshwater crayfish (Paranephrops planifrons) by LC/MS
- In Australia, 22 µg/kg NOD was detected in prawn by LC-MS/MS (Van Buynder at al 2001) while no toxin was detected in freshwater shrimp by LC-MS/MS (Stewart et al 2012).

The accumulation of NOD was also reported in soft tissue of clams (*Macoma balthica*) up to 130 µg/kg dw from the Gulf of Riga and the estuary of the River Daugava by ELISA, the lack of information on the methodology do not permit using these data for risk assessment (Barda et al 2015).

2) Food supplements

Only one publication investigated the analysis of nodularins on food supplements. It was shown that these toxins were **not detected in 18 samples of *A. flos-aquae, Spirulina and Chlorella* products from the German market by PP1A, ELISA and LC-MS/MS** (Heussner at al 2012).

3) Edible crops

No data available.

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CYNs

1) Food of animal origin

In fish, 3 publications are available but **none from European countries**:

- From Brazil, no CYN was detected in tilapia fish muscle by LC/MS (Galvao et al 2009);
- From Mexico, among the 9 species investigated, the highest CYN level was detected in the muscle of *Heterandria jonesii* with a mean of 1.26 ± 0.21 µg/kg fw by ELISA (Berry et al 2012);
- Niedzwiadek et al 2012 did not detect CYN in the muscle of different fish (artic char, catfish, trout, tilapia and salmon) from retail markets across Canada by ELISA.

There is no publication dealing with CYN accumulation in a shellfish species with reliable analytical method.

Only one publication with reliable analytical method (although semiquantitative) provides data on crustacean:

- Niedzwiadek et al 2012 did not detect CYN in shrimp from retail markets across Canada by ELISA.

2) Food supplements

Two publications have investigated the presence of CYN in food supplements:

- Heussner et al 2012 **did not detect CYN in 18 samples** of *A. flos-aquae, Spirulina and Chlorella* products from the German market by LC-MS/MS

- Similarly, Liu et al 2011 did not detect CYN in samples of A. flos aquae food supplements from the lake Klamath (Oregon, US) and chocolate containing BGA by HPLC-UV.

3) Edible crops

The accumulation of CYN in vegetables has been tested only through laboratory studies by Prieto et al 2011 and Kittler et al 2012. The results from the experiments of Prieto et al 2011 are not reliable considering the description of the analytical method performed. In the only remaining paper it was shown that CYN may accumulated in the leaves of different vegetables (*Brassica oleracea, Brassica juncea* and *Sinapis alba*) after few days of administration at the roots level, reaching up to 4.29 ± 1.48 µg/kg of CYN measured by LC/MS-MS (Kittler et al 2012).

STXs

1) Food of animal origin

The highest worldwide concentrations reported in fish muscle from field studies are:

- 30.6 ± 14.5 µg/kg of PSP toxins in C*ichlidae* (*Geophagus brasiliensis*) from Brazil (Clemente et al. 2010) by HPLC-FD;
- 19.7 ± 9.3 µg/kg of STX eq (GTX 3 and 5) in Cichlidae (*Geophagus brasiliensis*) from Brazil (Clemente et al 2010) by HPLC-FD.

From retail markets across Canada, no PSP toxins were detected nor in shrimp nor in 5 types of fish muscle (artic char, salmon, trout, catfish and tilapia) by HPLC-FD (Niedzwiadek et al 2012).

Regarding European countries, no data are available.

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The study of Galvao et al (2009) showed a depuration of the PSP toxins in fish muscle as the level of 20 µg/kg dcSTX in tilapia decreased below the detection limit after only 5 days of depuration.

2) Food supplements

Regarding European countries, only one publication is available from the German market**: PSP toxins were not detected in 18 samples** of *A. flos-aquae, Spirulina and Chlorella* products by LC-MS/MS (Heussner et al., 2012).

3) Edible crops

No data are available.

BMAA

1) Food of animal origin

- In China, Jiao et al 2014 followed the accumulation of BMAA during a bloom occurrence from emergence to decline in the muscle of 11 fish species by LC/MS-MS. BMAA was detected in all the samples: on the emergence of the bloom, the fish *Parabramis pekinensis* showed the highest value of 3770 ± 4 020 µg/kg dw of BMAA; during the outbreak, the level rised up to 35910 ± 13 400 µg/kg dw of BMAA in *Erythroculter ilishaeformis*; on the decline of the bloom, the highest value was reported in *Cyprinus carpa* with 15740 ± 5421 µg/kg dw of BMAA.
- In US, Mondo et al 2012 have investigated the level of BMAA in great hammerhead shark (*Sphyrna mokarran*) by HPLC-FD: 5 800 ± 4 100 µg/kg of BMAA. Brand et al 2010 have reported the level of BMAA found in the muscle of 18 fish species in Florida by LC/MS-MS: the level of total BMAA ranged from not detected to 3 559 µg/kg of BMAA in bowfin (*Amia calva*) from the Caloosahatchee River.
- In Europe, Jiang et al 2014 detected up to **20 µg/kg of BMAA in the muscle of plaice** (*Pleuronectes platessa*) **and baltic herring** (*Clupea harengus*) while **no toxin was detected in cod, salmon and perch** by LC/MS-MS.
- Lage et al 2015 published data on the muscle of 11 fish species (trout, burbot, common rudd, eel, tench, ruffe, pike, pike-perch, roach, perch, bream) from a lake in Sweden: **no quantifiable peak was obtained from trout, burbot, eel and common rudd samples**; the highest value was detected in **pike-perch** *(Sander lucioperca*) with **6.42 ± 2.53 µg/kg dw of BMAA** by UPLC/MS-MS
- From several European countries (France, Sweden and Greece), Jiang et al 2014 detected **up to 900 µg BMAA /kg** in **oyster** raw soft tissue by LC/MS-MS (method fully validated) but there is only few samples which have been tested (only 1) in France and Greece.
- Masseret et al 2013 reported in the Thau lagoon (France) a level of **3300 ± 1 400 µg/kg of BMAA and 1 100 ± 350 of DAB in mussel and 1 300 ± 190 µg/kg µg/kg of BMAA and 1 300 ± 190 µg/kg of DAB in oyster** by LC/MS-MS. No modification of the DAB concentration was observed along the time period while the BMAA concentration was higher for both mussel and oyster during summer.
- Réveillon et al 2014 in the same French lake detected a very high level **in mussel and oyster with 14 000 and 10 000 µg/kg dw of total BMAA** respectively, with **9 700 and 8800 µg/kg dw of total DAB and 1 400 and 1 700 µg/kg dw of total AEG**. In mussels, the concentration is time-dependent with an increase during summer while in oyster the level was more stable.
- In US, the level of BMAA in oysters from Louisiana and Mississipi reached up to 17 100 µg/kg by LC/MS-MS (Christensen et al 2012) and higher levels up to 305 000 µg/kg of BMAA in

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oyster mantle muscle as well as 261 000 µg/kg of BMAA in paper pondshell mussel by LC/MS-MS (Brand et al 2010).

- In China, two mollusk (*Bellamya aeruginosa* and *Anodonta woodiana*) were investigated with a level reaching 3 550 ± 1 113 µg/kg dw of BMAA in the soft body by LC/MS-MS (Jiao et al 2014).
- Niedzwiadek et al 2012 did not detect BMAA in shrimp from retail markets across Canada by HPLC-FD;
- from Florida (US), Banack et al 2015 detected 400 µg/kg dw of free BMAA and 11 000 µg/kg dw of bound BMAA in the tail of spiny lobster and 1 100 and 400 µg/kg of free and bound DAB respectively by LC/MS-MS. They reported also the levels after boiling: 27 000 and 12 000 µg/kg dw of free and bound BMAA in the flesh boiled; Christensen et al 2012 reported a mean level of 8 700 µg/kg of BMAA in the adductor muscle of blue crab by LC/MS-MS; Brand et al 2010 investigated several species in Florida by LC/MS-MS and detected up to 3 042 µg/kg of BMAA in pink shrimp, up to 6 976 µg/kg of BMAA in blue crab while no toxin was detected in spider crab.
- In Nebraska (US) reservoirs, Al-Sammak et al 2014 reported values of free and bound BMAA as well as free and bound DAB for several fish species (carp, bass, white crappie, catfish, shad, walleye, wer) ranging from not detected up to 2 570 µg/kg fw of bound BMAA, up to 416 µg/kg fw of free BMAA, up to 1530 µg/kg fw for bound DAB and up to 364 µg/kg fw for free DAB by HPLC-FD.

In Europe, Jiang et al 2014 detected up to **460 µg/kg of BMAA in cooked shrimp** (*Caridea*) but **did not detect BMAA in cooked crayfish** (*Astacus leptodactylus*) by LC/MS-MS.

No data are available for BGAS and edible crops.

CTXs

1) Food of animal origin

There is only one publication referring to Lausake and Wiana (South Pacific Ocean, Republic of Vanuatu) which reported CTX level from a surgeon fish (*Ctenochaetus striatus*) flesh 4.8 ± 2 µg/kg of PCTX 3C eq using the RBA (Laurent et al 2011). This publication refers also to the publication of Chinain et al., (2010) reporting a level up to 5.58 µg/kg of PCTX 3C eq in the flesh of surgeon fish (*Scarus altipinnis*) using the RBA.

No data are available for occurrence of CTX in BGAS and edible crops.

LPS, lyngbyatoxins, palytoxins

No data are available for the occurrence of these toxins in food when produced by cyanobacteria.

3.1.3. International/national regulations/guidelines or "health alert" levels

This section refers to the information collected regarding regulation of cyanotoxins in food, food supplements or drinking water, also available in the data inventory presented in Appendix C in tabular format, as MS Excel spreadsheets, which can be downloaded from the EFSA website in a Microsoft Excel format together with this report. In the absence of formal regulation, some countries have adopted guidelines or alert levels.

Regulation/guidelines/health alert levels of cyanotoxins in food

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Very few countries have set guidelines or acceptable levels for cyanotoxins in food, and only in fish and shellfish. The only exception concerns saxitoxin in bivalve shellfish since it is already covered by regulations in Europe, USA, Canada, Australia and New Zealand for marine biotoxins (saxitoxins can by produced both by marine dinoflagellates and by freshwater cyanobacteria).

In the US, some States have health advisories in case of fish contamination by microcystins (do not eat advisory in Illinois and Ohio, clean fish well and discard guts in Washington) and sometimes also by anatoxin-a, cylindrospermopsin and saxitoxin (California, Washington).

Victoria State (Australia) defined acceptable levels of cyanobacterial toxins in seafood based on the work of a scientific advisory group that carried out a risk assessment regarding commercial and recreational seafood safety in the Gippsland Lakes. The seafoods of concern were fish, prawns and mussels harvested from the lakes. The identified toxins for the risk assessment were microcystins, nodularin, saxitoxins and cylindrospermopsin (Mulvenna et al., 2012).

Table 3.1.3.1. Health guideline values for cyanobacterial toxins in seafood (based on consumption by 2–16 year age group), Victorian Department of Health.

	Health guideline value (µg/kg of whole organism sample)		
Toxin	Fish	Prawns	Mussels or Molluscs
Cylindrospermopsin and deoxyCYN	18	24	39
Microcystin-LR* or variants plus Nodularin	24	32	51
Saxitoxins	800	800	800

* The guideline value represents the sum of all mycrocystins variants and nodularin ones.

The guideline values for CYN are based on a NOAEL of 30 µg/kg bw/day, and high level seafood consumption from Australian surveys. Uncertainty factors are 10 for intraspecies variability, 10 for interspecies variability and an additional factor of 2 given that there is recent evidence that CYN has teratogenic and reproductive effects, and there is preliminary evidence that it may be carcinogenic.

The guideline values for MCs are based on a NOAEL of 40 µg/kg bw/day, and high level seafood consumption from Australian surveys. Uncertainty factors are 10 for intraspecies variability, 10 for interspecies variability, and an additional factor of 2 for limitations in data, including evidence of tumour promotion, suspicion of carcinogenesis, conflicting data in teratogenesis, and recent evidence of reproductive toxicity.

In France, the National food safety agency issued some recommendations in case of fish contamination by microcystins or saxitoxins (AFSSA 2008, 2009).

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Table 3.1.3.2. High level seafood consumption (97.5th percentile), Victorian Department of Health.

Age group (years)	Commodity	Consumer intake [4] g/kg bw[5]/day	g/day	Survey[1,2]
≥17	Fish (diadromous and freshwater)[3]	5.1	377	1995
	Prawns	5.1	377	
	Mussels	2.4	178	
2-16	Fish (diadromous and freshwater)[3]	8.4	319	2007
	Prawns	6.2	236	
	Mussels	3.9	148	

Notes: [1] 1995 National Nutrition Survey of Australia (1995 NNS), surveying 13858 people aged 2 years and over. This survey used one 24-hour recall for all respondents and a second 24-hour recall for approximately 10% of respondents; [2] 2007 Australian National Children's Nutrition and Physical Activity Survey, surveying 4487 people aged 2–16 years. The survey used two 24-hr recalls for all respondents. For the purposes of estimating acute dietary exposures only a single day 24-hour recall is used; [3] Diadromous and freshwater fish consumption excluding all marine fish; [4] The food consumption data outlined in the table includes the amount consumed alone and as an ingredient in mixed foods. In DIAMOND, FSANZ's dietary exposure assessment computer program, all mixed foods have a recipe and these recipes were used to break down mixed foods into their raw commodity components. For example fish contained in a fish casserole are included in fish consumption; [5] Bodyweights: 74 kg > 17 years; 38 kg 2–16 years; [6] Consumption amounts for molluscs were used where there were insufficient consumers of mussels alone to derive a valid 97.5th percentile consumption value. There were only 11 consumers of mussels in the 2–16 year age group dataset. "Molluscs" includes mussels, octopus, oysters, scallops and squid.

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Table 3.1.3.3 : overview of the regulations/guidelines for cyanotoxins in food included in the inventory

Country	Food	Description of the regulation/recommendation	value	type[2]	Source
European countries					
All	Bivalve molluscs	Paralytic Shellfish Poisoning (PSP)	800 µg/kg (*saxitoxin equivalent*)	S	Regulation (EC) No 853/2004
Denmark	Bivalve molluscs	There are many sites in Denmark where blue mussels (*Mytilus edulis*), common cockle (*Cardium edule*), surf clam (*Spisula* spp.), scallop (*Pecitinidae* spp.), and oysters (*Ostrea edulis*) are produced and harvested commercially for consumption. The harvested mussels as well as the harvesting sites are monitored weekly, specifically for toxic algae and cyanobacteria (including species of Nodularia and Anabaena) and algal toxins (including cyanotoxins) since 1990. If concentrations are found (determined by HPLC or LC-MS analysis and mouse tests) that exceed the accepted levels, the harvesting site is closed off, and the harvested mussels are destroyed. A closed harvesting site is re-opened when two samples taken at least 48 hours apart show that toxin levels have decreased to acceptable levels again. The surveillance of the mussel production and harvesting sites is run by the Danish Veterinary and Food Administration.			Chorus, 2012
France	Fish	Microcystins: Based on the WHO Tolerable Daily Intake for chronic exposure of 0.04 µg/kg bw/day, a default body weight of 60 kg, a daily consumption of fish of 86 g (high percentile in the French database) and an intake of water (2 L at 1 µg/L), the limit for adults would be 5.6 µg MCs/kg of fish (or edible part of fish). For children, this value would be 1.4 µg/kg of fish (default body	5.6 µg MCs/kg fish for adults 1.4 µg MCs/kg fish for	R of AFSSA (2008).	Chorus, 2012; AFSSA 2008, 2009

[2]GV: guidance value, S: standard; HA: Health advisory, MAC: maximum acceptable concentration, MAV: maximum allowable value, AL: alert level; R: Recommendation

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		weight of 10 kg and daily consumption of 57 g). If data are available to show the absence of MCs in drinking water, the limits become 28 and 7 µg/kg of fish, respectively.	children		
	Fish	Saxitoxins	Ban of fish consumption if SXT is detected in water or if there is a bloom of STX producing cyanobacteria	R of AFSSA (2009).	
Non-European countries					
Australia	Shellfish	Saxitoxins	800 µg/kg	S	Food Standards Australia New Zealand (FSANZ) Food Standard 1.4.1
Australia: Victoria and New South Wales states	Fish	Microcystins and nodularin (sum value of all microcystins and nodularin present)	24 µg/kg	GV	Chorus, 2012; Mulvenna et al 2012
		Cylindrospermosin and deoxyCYN	18 µg/kg	GV	
		Saxitoxins	800 µg/kg	S	
	Prawns	Microcystins and nodularin (sum value of all microcystins and nodularin present)	32 µg/kg	GV	
		Cylindrospermosin and deoxyCYN	24 µg/kg	GV	
		Saxitoxins	800 µg/kg	S	

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	Mussels or molluscs	Microcystins and nodularin (sum value of all microcystins and nodularin present)	51 µg/kg	GV	
		Cylindrospermosin and deoxyCYN	39 µg/kg	GV	
		Saxitoxins	800 µg/kg	S	
Canada	Bivalve shellfish	Paralytic Shellfish Poisoning toxins (PSP)(saxitoxin equivalents)	800 µg/kg	S	http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/contaminants-guidelines-directives-eng.php
	Natural Health Products Ingredients Database	Microcystins : Classified as an NHP under Schedule 1, item 2 (an isolate) of the Natural Health Products Regulations			http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/contaminants-guidelines-directives-eng.php
		Microcystins in *Aphanizomenon flos-aquae* (LR or total) powder, non medicinal purposes : Colour Additive , Skin-Conditioning Agent - Emollient , Surfactant - Foam Booster. Route of adminidtration; oral (color additive) or topical (Skin-Conditioning Agent - Emollient).	1 mg/kg	MI restriction	http://webprod.hc-sc.gc.ca/nhpid-bdipsn/ingredReq.do?id=14563&lang=eng
	Natural health products (NHPs) containing the cyanobacterium Aphanizomenon flos-aquae	Microcystin LR in finished product	0.02 µg MC-LR/kg body weight/day	Tolerance Limit	http://www.hc-sc.gc.ca/dhp-mps/consultation/natur/consult_quality-qualite-eng.php
	Natural Health Products Ingredients Database	Microcystins in *Aphanizomenon flos-aquae* extract (LR or total)	1 mg/kg	MI restriction	http://webprod.hc-sc.gc.ca/nhpid-bdipsn/ingredReq.do?id=14563&lang=eng
	Natural Health Products Ingredients	*Spirulina* (no restriction for microcystins)			http://webprod.hc-sc.gc.ca/nhpid-bdipsn/ingredsReq.do?srchRchT

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	Database				xt=spirulina&srchRchRole=-1&mthd=Search&lang=eng
	Natural Health Products Ingredients Database	*Spirulina*, in raw material for microcystins	1 mg/kg	Tolerance limit	Health Canada, NHP, 2015
	Fish	Alberta: Consider limiting human consumption of whole fish and fish trimmings from this lake, as it is known that fish may store toxins in their liver. (People can safely consume fish fillets from this lake).		Health Advisory	Alberta health services, http://www.albertahealthservices.ca/assets/news/advisories/ne-pha-2015-06-25-bga-nz-thunder-lake.pdf
California	Fish	Microcystins	10 ng/g ww		Butler et al, 2012
		Cylindrospermosin	70 ng/g ww		
		Anatoxin-a	5000 ng/g ww		
Washington	Fish	Tier 1: Bloom Forming ‣ Scum Visible ‣ Before results ‣< 6 µg/L microcystin ‣< 1 µg/L anatoxin-a ‣< 4.5 µg/L cylindro ‣< 75 µg/L saxitoxin	CAUTION, clean fish well and discard guts	GV	Traine and Hardy 2015
		Tier II: ‣>6 µg/L microcystin ‣>1 µg/L anatoxin-a ‣>4.5 µg/L cylindro ‣>75 µg/L saxitoxin	WARNING, clean fish well and discard guts		
		Tier III: report of illness, pet death	DANGER, lake closed		

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Oregon	Fish	At this time, there is insufficient information to determine the risk of consuming fish caught in waters with a cyanoHAB. Studies have shown that toxins mainly accumulate in the liver and viscera of fish, and microcystin has been detected in the fillet (Vasconcelos, 1999; de Magalhaes et al., 2001; Kann, 2008; Washington Department of Ecology, 2010; Kann et al., 2011). At a minimum, organs and skin should be removed and discarded prior to cooking fillets and caution should be taken with shellfish as cyanotoxins have been shown to accumulate in edible tissue (Vasconcelos, 1999).			Oregon Health Authority 2015
Ohio	Fish	Microcystin LR (DO NOT EAT advisory if > GV)	28 µg/kg fish filet	GV	Ohio Fish Consumption Advisory Chemicals: (ODH 10/25/99)
Illinois	Fish	Microcystins (DO NOT EAT advisory if > GV).	28 µg/kg fish filet	GV	Illinois EPA 2012
Oregon	food supplement (BGAS)	Microcystins (MC-LR eq)	1 µg/kg DW	S	Gilroy et al (2000); Dietrich and Hoeger (2005) This level was adopted by the Oregon Health Division as a provisional regulatory standard for BGAS products in 1997
Nebraska	fish	Advises the public not to eat whole fish from health alert lakes	if total MCs > 20 µg/l	Health Advisory	Walker et al., 2008

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Regulation/guidelines/health alert levels of cyanotoxins in Food supplements

Table 3.1.3.4 summarises the European and US regulation concerning research, production and market introduction of micro-algal products (food supplements) according to the report prepared by JRC (Enzing et al. 2014).

Table 3.1.3.4: EU and US regulation concerning research, production and marketing of micro-algal products (including GM micro-algae) for food and feed applications

	Europe	United States
Research	- EC directives 2009/41/EC (contained use of GM algae) - EC directive 2001/18/EC (deliberate release of GM algae)	- NIH rDNA Guidelines - EPA Standards for microbiological practices, under the Toxic Substances Control Act (TSCA)
Production	- EC directives 2009/41/EC (contained use of GM algae) - EC directive 2001/18/EC (deliberate release of GM algae)	- TSCA Environmental Release Application (TERA) - Microbial Commercial Activity Notice (MCAN) under TSCA [- USDA Plant Protection Act]
Market introduction	- EC Regulation on Food Safety (EC 178/2002) - EC Regulation on Novel Foods and Novel Food Ingredients (EC 258/97) - EC Regulation on Genetically Modified Food and Feed (EC 1829/2003) - EC Regulation on traceability and labelling of GMOs and of food and feed products produced from GMOs (EC 1830/2003) - EC Regulation on Nutrition and Health claims made on foods (EC 1924/2006)	- Food, Drug and Cosmetic Act - Dietary Supplement Health and Education Act

In Europe, market introduction of food products using the whole microalgae organisms (such as *Spirulina*, or *Chlorella*) or products that include the micro-algae (like pasta with the green algae colour) are subject to the European Community Regulation on Food Safety (EC 178/2002).
If a product was on the market as a food or food ingredient and consumed to a significant degree before 15 May 1997, like *Arthrospira platensis*, its access to the market is not subject to the Novel Food Regulation (EC) No. 258/97. However, other specific legislation may restrict the placing on the market of this product as a food or food ingredient in some EU Member States (see table 3.1.3.1. for example regarding health claims).

In the US, *Spirulina* has been recognized as GRAS[3] under the "intended conditions of use" implying that it is "for use as an ingredient in foods, at levels ranging from 0.5 to 3.0 grams per serving", i.e. in relatively small amounts. Nevertheless, considering that safety level and possible hazards for consumption of *Spirulina* and *Spirulina*-related products have not been established beyond doubt, special precautionary measures would be necessary on the consumption of *Spirulina* products to some segments of the population at risk to include pregnant women, nursing mothers, people in dialysis and immune-compromised.

[3] "GRAS" is an acronym for the phrase Generally Recognized As Safe. Under sections 201(s) and 409 of the US Federal Food, Drug, and Cosmetic Act (the Act), any substance that is intentionally added to food is a food additive, that is subject to premarket review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use, or unless the use of the substance is otherwise excluded from the definition of a food additive http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/

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Two USA laws are applicable on micro-algae based food and feed products once they are sold on the consumer market:

- the Federal Food, Drug and Cosmetic Act (FD&C) introduced in 1938, which regulates all foods and food additives;
- the Dietary Supplement Health and Education Act (DSHEA) introduced in 1994, which amended the FD&C Act to cover dietary ingredients and supplements.

The FDA regulates both acts. The FDA Center for Food Safety and Applied Nutrition (CFSAN) is responsible for regulating food ingredients and ensuring that those ingredients are safe and lawful. The authorisation of feed products falls under the FDA Center for Veterinary Medicine (CVM)

Under US regulations *Spirulina* is a "grandfathered" dietary ingredient because it was legally marketed before 1994 (DSHEA, 1994). Accordingly, organized clinical dose-escalation studies to observe safety profile are not required by US law for spirulina-based supplements.
The Dietary Supplements Information Expert Committee (DSI-EC) of the United States Pharmacopeial Convention (USP) unanimously voted for a Class A safety assignment for *S. maxima* and *S. platensis*, indicating that the available evidence does not indicate a serious risk to health or other public health concern that precludes admission of quality monographs into the United States Pharmacopeia and National Formulary (USP–NF) when these dietary ingredients are properly identified, formulated, and used. USP is developing a quality monograph for spirulina. The Committee determined that the proposed monograph would need to include a method to test for the presence of microcystins and that the microcystin content should be limited to NMT 1 ppm based on a review of the available safety literature (Marles et al., 2011).
Although historically *Spirulina* was used as a food or food component, no quality monograph is available (Marles et al., 2011). Some authors have suggested the need for quality standards for *Spirulina* (Rellan et al., 2009; Eisenbrand et al., 2008).

Similarly to the provisional WHO-guideline for drinking water, Gilroy et al. (2000) calculated a TDI of 0.04 µg MC-LR/kg bw/day based on the MC-LR mouse NOAEL of 40 µg/kg defined by Fawell et al. (1999) and the application of a total of 1000-fold uncertainty factor, which resulted in a provisional tolerable level for MCs in BGAS of 1.0 µg MC-LR/g dry weight. This level was adopted by the Oregon Health Division as a provisional regulatory standard for BGAS products on 23/10/1997. However, as this provisional tolerable level was calculated for an adult only, children (e.g., 10–20 kg body weight) would be exposed to three to six times the MC-LR equivalent concentration per day. Thus, in the worst case (1.0 µg MC-LR/g dw BGAS and daily intake of 10 g BGAS), children could ingest up to a 30-fold amount of MC-equivalents than that considered safe by the Oregon Health Division over weeks, months or even years. More conservative guidance values for MC content as well as a restriction for application to infants and children would be advisable (Dietrich and Hoeger, 2005).

Health Canada issued a review of toxins in blue-green algae (Cyanobacteria) in 2008. According to Marles et al. (2011) the review stated that "consumers can safely use products made only from *Spirulina* blue-green algae as these were found to be free of microcystins." The review also noted that long-term ingestion of microcystins from the toxic cyanobacterial species *M. aeruginosa* can cause liver damage. Children potentially are at risk of exposure to harmful concentrations of toxins if they ingest cyanobacterial products for an extended period of time. As a precaution, Health Canada recommended that the use of non-spirulina cyanobacteria by children be discontinued until further studies have been conducted (Health Canada, 2008)[4].

In Australia, the Therapeutic Goods Administration changed the regulatory status of *S. maxima* and *S. platensis* in May 2005 from "excipient only" to "active ingredient". The most up-to-date reference on *S. maxima* and *S. platensis* stated that the correct taxonomic designation of the genus for the two species were *Arthrospira* and not *Spirulina*. The Herbal Ingredient Names Committee (an internal TGA

[4] Health Canada (2008). Blue green algae (Cyanobacteria) and their toxins. Internet: http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/cyanobactereng.php (accessed by Marles et al 22 October 2009 but the link is not valid anymore).

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naming committee) determined that the correct Australian Approved Names for these two algae should be *Arthrospira platensis* and *Arthrospira maxima*, but the committee allows the use of the more frequently used term spirulina elsewhere on the label (TGA, 2005[5]; Marles et al., 2011).

At the international level, the *Codex Alimentarius* Commission rejected in 2003 the proposal that microalgae should be regarded as food because of the lack of history on their safe use (*Codex Alimentarius* Commission 2003; Gutiérrez-Praena, 2013).

Regulation/guidelines/health alert levels of cyanotoxins in drinking water

The World Health Organization (WHO) released a provisional drinking water guideline of 1 µg/L for microcystin-LR, excluding other known cyanotoxins since there was insufficient data to derive guideline values for these toxins (WHO, 1998; WHO, 2003).

A 13-week study in mice with microcystin-LR (Fawell et al., 1994) was considered the most suitable for the derivation of a guideline value. In this study, a NOAEL of 40 µg/kg bw/day was determined for liver pathology. A TDI of 0.04 µg/kg bw/day was calculated by applying an uncertainty factor of 1000 (100 for intra- and interspecies variation, 10 for limitations in the database, in particular lack of data on chronic toxicity and carcinogenicity) to the NOAEL. An allocation factor of 0.80 was used for the proportion of daily exposure arising from drinking-water, because there is little exposure from any other source and route. The resulting guideline value for **total microcystin-LR (free plus cell-bound**) is 1 µg/litre (rounded figure) in drinking-water.
The guideline value thus calculated is supported by a 44-day study in which pigs were exposed, in their drinking-water, to an extract from *M. aeruginosa* containing microcystin-LR.
The guideline value of 1 µg/litre for microcystin-LR is provisional, as the database is limited, new data for the toxicity of cyanobacterial toxins are being generated, and the guideline value covers only microcystin-LR (WHO, 2003).

Many countries around the word followed the WHO guidance and use the 1 µg/L in drinking water as a standard or a guidance value in their national regulation, in Europe (Czech Republic, France, Finland, Italy, Portugal, Spain), Australia, Brazil, Singapore, Uruguay, South Africa. Canada set its own maximum acceptable concentration at 1.5 µg/L for MC-LR. Many other countries have no limit or guidance for cyanotoxins in drinking water: in Europe (Denmark, Estonia, Germany, Greece, Hungary, Ireland, Latvia, Luxembourg, the Netherlands, Poland, Romania, UK), and outside such as Argentina, Cuba. According to Dietrich and Hoeger (2005) MCs have been reported in final water in Argentina, Australia, Bangladesh, Canada, Czech Republic, China, Finland, France, Germany, Latvia, Poland, Thailand, Turkey, Spain, Switzerland and USA.
Some countries also set guidance values for other cyanotoxins such as nodularin, cylindrospermopsin and saxitoxin (Australia, Brazil, New Zealand).

In the US, there are currently no federal water quality criteria, or regulations for cyanobacteria or cyanotoxins in drinking water under the Safe Drinking Water Act (SDWA) or in ambient waters under the Clean Water Act (CWA)[6]. However, several states do have various values that serve as guidelines or thresholds for certain management actions: California, Florida, Ohio, Oregon, and Minnesota.
In June 2015, US-EPA released drinking water Health Advisories for microcystins and cylindrospermopsin. In 2015 lso Health Canada published updated Guideline for Drinking Water[7].

[5] TGA. (2005). Therapeutic Goods Administration, Australia. Complementary Medicines Evaluation Committee 52nd meeting. August 2005. Internet: http://www.tga.gov.au/docs/pdf/cmec/cmecmi52.pdf
[6] http://www2.epa.gov/nutrient-policy-data/guidelines-and-recommendations
[7] ANSES, 2015; Butler et al, 2012 ; Chorus, 2012; Farrer et al, 2015 ; Health Canada, 2002; Minnesota Department of Health, 2012 ; US-EPA, 2015.

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Table 3.1.3.5 : Regulation/guidelines/health alert levels in drinking water

Country	Description of the regulation/recommendation	value	type[8]	Source
For all	microcystin-LR	1.0 µg/L	provisional GV	WHO, 1998, 2003
European countries				
Austria	No answer provided to the survey conducted by ANSES (2015)[9]			
Belgium				
Bulgaria				
Croatia				
Cyprus				
Czech Republic	Cyanobacteria in raw water (as cell counts or biomass or concentration of chlorophyll-a)	3 levels		Chorus, 2012; ANSES, 2015; Decree of the Ministry of Health No 252/2004 Coll. as amended (on hygienic requirements on drinking water and hot water and on scope and frequency of water monitoring).
	Microcystin-LR in treated water (monitored once per week)	1 µg/L	S	
Denmark	Cyanotoxins/microcystins are not regulated in drinking water in Denmark. It is not relevant to regulate for these toxins as we only use groundwater for drinking water.			ANSES, 2015[10]
Estonia	Cyanotoxins are not regulated in drinking water, as most of the drinking water			ANSES, 2015

[8]GV: guidance value, S: standard; HA: Health advisory, MAC: maximum acceptable concentration, MAV: maximum allowable value, AL: alert level

[9] ANSES, 2015. Survey on regulation of cyanotoxins in drinking water (email consultation to ENDWARE, from February 5 to March 2, 2015. ENDWARE (European Network of Drinking Water Regulators) is an informal group of representatives of ministries or public health agencies from European countries and a representative of the European Commission (DG Environment). This group meets twice a year since 1995.

[10] In Chorus, 2012, it is stated that "No specific cyanotoxin drinking water regulation as almost all drinking water originates from ground water (99%). In specific cases where surface waters is used to supplement drinking water supply in dry season, the provisional WHO guideline for Microcystin-LR of 1 µg/L is applied if needed". However this information has not been confirmed in our survey conducted in March 2015. The representative of the Danish Ministry of Environment has indicated that "In the past approximately 1% of our drinking water was produced from surface water, but that's not the case anymore. For at least the last five years all our drinking water originates from groundwater. The information in the booklet on from where our drinking water originates is no longer correct. I therefore believe that the text from the booklet regarding Denmark should not be stated in the summary, as it no longer is correct".

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Country	Description of the regulation/recommendation	value	type[8]	Source
	comes from ground water.			
Finland	Cyanobacteria in raw water	3 levels	GV	
	Microcystins (sum of all variants) in raw water • Identify cyanobacteria and toxin concentrations in drinking water • Potential health risks are assessed by municipal health protection authority • Ensure the effectiveness of water treatment processes, e.g. enhance coagulation/clarification or activated carbon filtration • Change water intake site, depth or source Microcystins (sum of all variants) in raw water • Identify cyanobacteria and toxin concentrations in drinking water • Potential health risks are assessed by municipal health protection authority • Ensure the effectiveness of water treatment processes, e.g. enhance coagulation/clarification or activated carbon filtration • Change water intake site, depth or source	If > 1µg/L	GV	Chorus, 2012
	Microcystins (sum of all variants) in drinking water • Identify cyanobacteria and toxin concentrations in incoming and drinking water • If the concentration exceeds the limit as a one-off occurrence, the decision on whether the water can continue to be used for drinking and cooking purposes is to be taken on a case-by-case basis • In the event that the concentration remains elevated for a sustained period of time, the use of water for cooking or drinking purposes will be prohibited and the facility will organise the distribution of alternative water supplies to the affected population • Change water intake site, depth or source • Enhance water treatment processes	If > 1 µg/L	GV	
	Microcystins (sum of all variants) in drinking water • Identify cyanobacteria and toxin concentrations in incoming water and drinking water • Water not to be used for domestic purposes except for toilet flushing	If > 10 µg/L	GV	
France	Microcystins (sum of all variants), analysis required in raw water and at the point	1 µg/L	S	Chorus, 2012 ; Order of 11

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Country	Description of the regulation/recommendation	value	type[8]	Source
	of distribution (drinking water) only when cyanobacteria proliferate (visual observation and/or analytical results). Water used in food industry (in case its water supply is not from the public network) is covered by another order. Microcystins are not listed in the parameters to be analyzed.			January 2007
Germany	No specific cyanotoxin regulations as only about 20% of water supply is from surface water, and that mostly from well protected reservoirs. However, for nonregulated chemicals the Drinking-water Ordinance requires that they do not occur in hazardous concentrations. On this basis, where cyanobacteria do occur, the WHO GV can be applied for microcystins. National guidance for substances with incomplete toxicological evidence proposes <0.1 µg/L if carcinogenesis cannot be excluded (until data are generated that allow higher levels), and this can be applied to Cylindrospermopsin.			Chorus, 2012; ANSES, 2015
Greece	No national regulation or guidance			Chorus, 2012
Hungary	The microscopical analysis of drinking water is required. The limit values of algae and cyanobacteria is 5 x 10^3 number/ L – in case of drinking water catchment from surface water or bank filtered water the limit value is one-tenth, any other cases one-fiftieth of this limit value. There is not limit value for microcystins nor cyanotoxins in drinking water.			Chorus, 2012 ; Governmental Decree No. 201 of 2001 (X.25.) on the monitoring and quality requirements of drinking water, Annex I, Table E
Ireland	Microcystins or other cyanotoxins are not regulated in drinking water			Chorus, 2012
Italy	National decree includes "algae" as accessory parameter to monitor if local authorities presume a risk, based on the provisional WHO GV for Microcystin-LR.			
	Microcystin-LR (sum of all variants in MC-LR equivalent), the detection of cyanotoxins in water intended for human consumption is not subject to routine controls or checks which occur at pre-arranged intervals, but must only be carried out in cases identified following a risk assessment	1 µg/L	S	ANSES, 2015
Latvia	Cyanotoxins are not regulated in drinking water, as the water supply is mostly based on groundwater (with only one exception for part of capital city Riga)			ANSES, 2015
Lithuania	No answer provided to the survey conducted by ANSES (2015)			
Luxembourg	Microcystins or other cyanotoxins are not regulated in drinking water			ANSES, 2015
Malta	No answer provided to the survey conducted by ANSES (2015)			

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Country	Description of the regulation/recommendation	value	type[8]	Source
Netherlands	No specific regulations for cyanotoxins in drinking water, although about 40% of water supply is from surface water, mainly from well protected reservoirs and infiltration basins. However, concentrations of micro-organisms may not exceed levels which may have adverse consequences for public health. For the production of drinking water, several steps in the treatment process are efficient barriers that prevent cyanobacterial cells and microcystins from reaching finished drinking water. In case this should happen, the Netherlands would apply WHO guidance, < 4700 cells/mL and for microcystins the WHO GV of 1µg/L.			Chorus, 2012; ANSES, 2015
Poland	The Guideline value of 1 µg/L of microcystin-LR has been excluded from Polish legislation by the Ministry of Health in 2007. The new regulation without a guideline value of microcystin was based on European Directive 98/83/EC which does not state such a requirement.However, some water treatment plants that have a surface water intake monitor the probability of microcystin occurrence on their own initiative and use the WHO GV for microcystin-LR of 1 µg/L.			Chorus, 2012
Portugal	Microcystin-LR, must be controlled at the exit of the water treatment plant everytime there is something suspicious in the surface water source. If the cyanobacteria are above 2000 cells/L, the number of samples in the operational monitoring should be increased.	1 µg/L	indicator parameter	ANSES, 2015
Romania	Microcystins or other cyanotoxins are not regulated in drinking water			ANSES, 2015
Slovakia	No answer provided to the survey conducted by ANSES (2015)			
Slovenia	No answer provided to the survey conducted by ANSES (2015)			
Spain	Microcystin shall be only measured at the drinking water treatment plant or at the water tower (reservoir) if there is a reason to suspect eutrophication in water source	1 µg/L	S	Chorus, 2012 ANSES, 2015
Sweden	Cyanotoxins are not regulated in drinking water			ANSES, 2015
United Kingdom	Regulation of the water supply (Water Quality) states that water must not contain any micro-organism or parasite or substance (other than a parameter) at a concentration or value which could constitute a potential danger to human health. However, there is no value for regulating cyanotoxins.			ANSES, 2015
Non-European countries				

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Country	Description of the regulation/recommendation	value	type[8]	Source
Argentina	Requirements neither for Risk Management Framework nor for cyanotoxin surveillance, but some water utilities have implemented either or both; these refer to the provisional guideline by WHO for Microcystin-LR of 1 µg/L			Chorus, 2012
Australia	Microcystin (toxicity equivalents of MC-LR): equivalent to 6500 cells/mL or a biovolume of 0.6 mm³/L of a highly toxic strain of *Microcystis aeruginosa*	1.3 µg/L	GV	Chorus, 2012
	Nodularin: HAL at 40 000 cells/mL or a biovolume of 9.1mm³/L of a highly toxic strain of *Nodularia spumigena*	no value		
	Cylindrospermopsin: equivalent to 15 000-20 000 cells/mL or a biovolume of 0.6-0.8 mm³/L of *Cylindrospermopsis raciborskii*	1 µg/L		
	Saxitoxins (toxicity equivalents to STX): equivalent to 20,000 cells/mL or a biovolume of 5 mm³/L of a highly toxic strain of *Anabaena circinalis*	3 µg/L		
	For livestock: > 11 500 cells/mL of *Microcystis aeruginosa* (or a trigger value of 2.3 µg/L toxicity equivalents of MC-LR): increasing risk to livestock health is likely. New South Wales state only (interim measure): alert level of 25 000 cells/mL for *Anabaena circinalis*			
	For irrigation: Victoria and New South Wales states recommend that water contaminated with blue-green algae should not be spray irrigated on vegetables and fruit, or come in contact with plants being grown for food.			
Brazil	Cyanobacteria	10^4 -20^4 cells/mL or 1 mm³/L biovolume	GV	Chorus, 2012[11]
	Microcystins	1 µg/L	S	

[11] In 2015 Health Canada published an update of the drinking water guidelines for cyanobacterial toxins, available at http://healthycanadians.gc.ca/publications/healthy-living-vie-saine/water-cyanobacteria-cyanobacterie-eau/index-eng.php and stating : *The maximum acceptable concentration (MAC) for the cyanobacterial toxin microcystin-LR in drinking water is 0.0015 mg/L (1.5 µg/L). This guideline is believed to be protective of human health against exposure to other microcystins (total microcystins) that may also be present.*

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Country	Description of the regulation/recommendation	value	type[8]	Source
	Cylindrospermopsin	15 µg/L	GV	
	Saxitoxin	3 µg SXTeq/L	GV	
Canada	Microcystin-LR, not a parameter with regularly scheduled monitoring frequencies, most municipal drinking water treatment plants monitor for algal toxins only when a bloom occurs.	1.5 µg/L	MAC	Chorus, 2012
	In Alberta: provincial regulations require all MCs regulated drinking water treatment plants drawing surface water to test for microcystin-LR during July-August which is the high risk bloom-period	1.5 µg/L	MAC	
	In Quebec: sum of all microcystins	1.5 µg/L	MAC	
	Anatoxin-a (only in Quebec)	3.7 µg/L	Provisional MAC	
Cuba	No regulatory requirements but framework tested in a pilot reservoir based on Cyanobacteria in raw water, scum, any report of toxic effect (humans or animals) For irrigation: restriction if the total number of phytoplankton> 100 000 cells/mL. Leafy vegetables irrigated with such water would be analysed for cyanotoxin contamination			Chorus, 2012
New Zealand	Cyanotoxins require monitoring when known to be present at health significant levels (50% of the MAV).			Chorus, 2012
	Microcystins (as MC-LR equivalents)	1 µg/L	Provisional MAV (PMAV)	
	Cylindrospermopsin	1 µg/L	PMAV	
	Saxitoxin (as STX equivalents)	3 µg/L	PMAV	
	Anatoxin-a	6 µg/L	PMAV	
	Anatoxin-a(s)	1 µg/L	PMAV	
	Homoanatoxin-a	2 µg/L	PMAV	
	Nodularin	1 µg/L	PMAV	
Singapore	A monitoring programme for Microcystin-LR was introduced in 2003, extended in 2006 to MC-RR, LA, YR, in 2009 to Cylindrospermopsin and in 2010 to Anatoxin-a	MC-LR 1 µg/L in free and cell-bound forms	S	Chorus, 2012
Turkey	Currently, no national guidelines			Chorus, 2012; ANSES, 2015

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Country	Description of the regulation/recommendation	value	type[8]	Source
	proposed but not yet implemeted: Cyanobacteria in raw water, monthly monitoring. If the threshold is exceeded, weekly sampling and toxins analysis	>5000 cells/mL or >1µg/L chlorophyll-a		
	proposed but not yet implemeted: Microcystins (sum of all MCs, as MC-LR equivalents) in raw water. If the threshold is exceeded, MC analysis in treated water	1 µg/L	S	
Uruguay (since 2011)	Microcystin-LR in treated drinking water (measured by the method ISO 20179:2005)	1 µg/L	S	Chorus, 2012
South Africa	Microcystin-LR	1 µg/L	GV	Chorus, 2012
United States of America	No national requirements, but action taken by many States			Chorus, 2012
California	Microcystins (LA, LR, RR, YR) for human	no value		Chorus, 2012; Butler et al., 2012
	Microcystins (LA, LR, RR, YR) for cattle	0.9 µg/L (sub-chronic) 50 µg/L (acute)	GV	
	Microcystins (LA, LR, RR, YR) for dog	2 µg/L (sub-chronic) 100 µg/L (acute)	GV	
	Cylindrospermopsin for human	no value		
	Cylindrospermopsin for cattle	5 µg/L (sub-chronic) 60 µg/L (acute)	GV	
	Cylindrospermopsin for dog	10 µg/L (sub-chronic) 200 µg/L (acute)	GV	
	Anatoxin-a for human	no value		
	Anatoxin-a for cattle	40 µg/L (sub-chronic & acute)	GV	
	Anatoxin-a for dog	100 µg/L (sub-chronic & acute)	GV	

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Country	Description of the regulation/recommendation	value	type[8]	Source
Florida	Microcystins	1 µg/L chronic; 10 µg/L 90 day	HAL	Chorus, 2012
Ohio	Microcystins	1 µg/L	MAC	Chorus, 2012[12]
	Anatoxin-a	20 µg/L	GV/AL	US-EPA 2015, http://www2.epa.gov/nutrient-policy-data/guidelines-and-recommendations
	Cylindrospermopsin	1 µg/L	GV/AL	
	Saxitoxin	0.2 µg/L	GV/AL	
Oregon	Microcystins	1 µg/L	GV	Farrer et al, 2015[13]
	Cylindrospermopsin	1 µg/L	GV	
	Anatoxin-a	3 µg/L	GV	
	Saxitoxin	1 µg/L	GV	
Minnesota	Microcytin LR	0.04 µg/L	GV/AL	Minnesota Department of Health 2012 ; D'Anglada 2014
US-EPA	Microcystins	0.3 µg/L for children < 6 years, 1.6 µg/L for children > 6 years and adults	HA (Health advisory)	US-EPA 2015
	Cylindrospermopsin	0.7 µg/L for children < 6 years, 3.0 µg/L for children > 6 years and adults	HA (Health advisory)	

[12] In 2015, Ohio adopted values as identified by USEPA also for MC (see below)
[13] information in Chorus (2012) for Oregon is not confirmed by Farrer et al (2015). Priority is given to Farrer et al (2015) since it is a paper from Oregon Health Authority

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Regulation/recommendations related to bathing water have been implemented/suggested in some European and non European countries, but this kind of actions were not considered atinent to this procurement.

3.2. Analytical Methods in biological media

Data coming from the ELS were divided in subsets, one for each cyanotoxin. Individual data are available in the inventory in Appendix C in tabular format, as MS Excel spreadsheets, which can be downloaded from the EFSA website in a Microsoft Excel format together with this report

3.2.1. Microcystins

Sample preparation

Food supplements: extraction and cleanup

Jiang et al. (2008) extracted MCs from *Spirulina* health food (tablets and capsules) obtained from various retail outlets in China with 75% aqueous MeOH. Ortelli and co-workers (2008) used the same solvent mixture and the same sample to solvent ratio (1 g BGAS/10 mL solvent) and applied the procedure to tablets and pills made of BGA. Lawrence and co-workers (2001) also used 75% aqueous MeOH to extract MCs from BGA products (consisting of *Aphanizomenon, Spirulina*, and unidentified blue-green algae) in the form of pills, capsules, and powders; so did Vinogradova et al. (2011).

Saker and co-workers (Saker et al., 2005; Saker et al., 2006) analysed dietary supplements to determine if they were toxigenic or not but the authors did not look for the presence of toxins; they extracted the DNA and tested the samples using a multiplex PCR. The extraction was performed with DNA extraction kits commercially available.

Jiang et al. (2008) reported the successive use of two types of SPE cartridges, a reversed phase ODS and a silica SPE to clean BGAS extracts and optimized the wash and elution conditions to have the best efficiency. Yet, the extraction procedure was not optimal for all tested toxins as the toxin recovery ranged from 40% for MC-YR to 82% for MC-RR.

Lawrence and co-workers (2001) purified the BGA extracts on C18 cartridges prior to their analysis. To ensure minimal influence of the sample on the MCs elution patterns and to avoid losses during the wash step, the authors limited the amount of matrix passed through the cartridges to 50 mg.

Vegetal matrices: extraction and cleanup

MCs can be extracted from lyophilized vegetables (lettuce, water spinach, cabbage, choi sum, carrot, turnip, potato, pumpkin, cucumber and eggplant) using a mixture of acidified aqueous methanol solution (methanol/water/TFA). The optimum composition of the extraction solvent mixture was found to be 80/19.9/0.1 (v/v/v) for MC-LR, MC-RR and MC-YR although better recoveries were obtained with 1% TFA for some toxin/vegetable combinations (Li et al., 2014).

Li et al. (2014) reported the use of SPE cartridges to purify the vegetable extracts prior to their analysis. Two different types of cartridges were used, a reversed-phase C18 for most of the vegetables mentioned above and a mixed-mode cartridge (HLB: hydrophilic-lipophilic balance) for cucumber and eggplant, as the recovery with C18 cartridges was poor for the latter vegetables.

Fish and shellfish: extraction and cleanup

Different extraction procedures have been applied to different types of samples to extract MCs, either as free or total toxins, the latter fraction being composed of the MCs bound to proteins and the free MCs.

Williams et al. (1997) extracted free MCs from mussels using 100% MeOH. The combined supernatants were evaporated to dryness and the oily residue was tested by PP-1 assay.

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Lawrence et al. (2001) extracted MCs from salmon, rainbow trout and pickerel using 75% MeOH. A similar approach was applied by Berry et al. (2011) and Suchy & Berry (2012) who extracted MCs from fish species (silversides, *Chirostoma* spp.; *Goodea* sp.; and carp, *Cyprinus carpio*) with 75% MeOH, followed by 75% MeOH with 0.05% acetic acid, with mechanical homogenization.

Preece and co-workers (2015) tested and compared four extraction procedures using different solvent mixtures: 75% ACN, 75%, 90% or 100% MeOH, on rainbow trout dorsal muscle tissue spiked with a mixture of MC-LR, RR, YR and LW. The best recoveries were obtained with 75% MeOH, however when applied to mussels, all the samples extracted with 75% MeOH resulted in false positives with the anti-Adda ELISA. According to the authors, this could be due to the fact that the extraction procedure is long (a few hours) and is likely to co-extract interfering compounds.

Cadel-Six et al. (2014) reported the extraction of MCs from different tissues of juvenile trouts (gills, liver, intestine, muscle) using a mixture of water:methanol:butanol (75:20:5) with a sample to solvent ratio of 10 mg of freeze dried tissue/1 mL solvent mixture.

MC-LR and its glutathione conjugates (MC-LR-GSH) can be extracted from fish tissues (liver and kidney) using 0.01 M EDTA–Na_2 (0.01 M) –5% acetic acid (Dai et al., 2008). The same extraction procedure has been applied to snails (*Bellamya aeruginosa*) and shrimps (*Macrobrachium nipponensis*) to extract MC-LR-GSH, MC-LR and its cysteine conjugate (MC-LR-Cys) (Zhang et al., 2009).

The use of organic solvents is not necessary as Bogialli and co-workers (2005) extracted MCs and NOD from trout, mullet, perch and goldfish samples using a stainless-steel column used as extraction cell. The fish tissues blended with sand were packed in the extraction cell and the toxins were extracted with water acidified to pH 2 (HCl) and heated at 80°C, passed through the cell at a constant flow rate.

For the extraction of total MCs from different matrices, a specific procedure has been described in the literature; this is the Lemieux oxidation, that enables the release of a portion of the Adda moiety as 2-methyl-3-methoxy-4-phenylbutanoic acid (MMPB).

Cadel-Six et al. (2014) used this procedure on trout juveniles that were digested in Sörensen's phosphate buffer using Trypsin and oxidised with 0.1 M $KMnO_4$ and 0.1 M $NaIO_4$, at pH 9.0 for 4 h, prior to LC-MS/MS analysis.

Suchy and Berry (2012) extracted total MCs with Lemieux oxidation using $KHCO_3$, $KMnO_4$ and $NaIO_4$, followed by methyl esterification, as a means to volatilize analytes for subsequent headspace solid phase micro extraction (SPME) sampling and GC-MS analysis.

Williams et al. (1997) determined the total MCs content of mussels using the oxidation procedure prior to GC-MS analysis. The authors reported important differences between free and total MCs with less than 0.1% of the total MC burden in the mussels found to be extractable with MeOH.

The use of SPE cartridges has been reported to clean different extracts prior to their analysis using various methods. Thus Cadel-Six et al. (2014) tested Strata X and Oasis hydrophilic-lipophilic balance (HLB) cartridge (60 and 30 mg respectively) and selected the Strata X cartridges. These authors also tested Oasis HLB (200 mg) and SampliQ OPT (150 mg) cartridges for the analysis of total MCs and reported that the latter cartridges were more efficient.

Different types of cartridges can be used in combination to increase the cleanup efficiency; this was the case for Oasis HLB and Sep-Pak silica gel cartridges used to purify extracts obtained from fish, snails and shrimps (Dai et al., 2008; Zhang et al., 2009).

Lawrence and Menard (2001) chose a somewhat different approach as they cleaned fish samples on immuno affinity columns bearing immobilized anti-MC-LR polyclonal antibodies. Average recoveries of the overall procedure ranged from 73% (MC-RR) to 87% for NOD

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Detection: chemico-analytical methods

LC-MS

There are several accounts in the literature of the use of LC-MS for the analysis of sample extracts to determine their MC content (Bogialli et al., 2005; Cadel-Six et al., 2014; Dai et al., 2008; Geis-Asteggiante et al., 2011a; Hiller et al., 2007; Karlsson et al., 2005; Kohoutek et al., 2010; Mekebri et al., 2009; Neffling et al., 2009; Ott and Carmichael, 2006; Wu et al., 2010).

The toxins are generally separated on reversed-phase columns of different characteristics (Bogialli et al., 2005; Cadel-Six et al., 2014; Dai et al., 2008; Preece et al., 2015). Although the mobile phase composition might vary, ACN is the preferred solvent and either or both mobile phases run in gradient mode are acidified with formic acid.

The MCs are analysed in positive ionization mode and can be detected as single- or double-charged precursor. Thus Cadel-Six et al. (2014) monitored the precursor ion m/z 995.8 for MC-LR ([M+H]+) and two characteristic fragments, m/z 135.3 (characteristic of ADDA fragment) and m/z 213.3.

Similarly, Dai et al. (2008) monitored the precursor ion [M+H]+ at m/z 995.5 for MC-LR. The reported product ions (m/z) included 599.3, 553.2, 866.4, 967.4, and 977.4. For MC-LR-GSH, the precursor ion was at m/z of 1302.8. The doubly protonated ion ($[M+ 2H]^{2+,}$ m/z 652.0) was also observed.

Bogialli et al. (2005) monitored several MC variants and the selected precursor ion for some of them was the double-charged ion (m/z 498 for MC-LR, m/z 520 for MC-RR).

After the Lemieux oxidation, the MMPB can be directly detected by LC-MS/MS with a precursor ion $[MMPB + H]^{+}$ m/z 209.3, and three characteristic fragments measured at m/z 91.1, m/z 131.1 and m/z 191.1, respectively (Cadel-Six et al., 2014).

LC-DAD

Lawrence and Menard (2001) analysed fish samples purified on IAC analysed by LC-DAD. The separation of the analytes was achieved on a C18 column with a gradient of mobile phases composed of water and ACN and acidified with TFA. The detection was at 238 nm and the authors reported that for BGA samples, LC-DAD results were comparable to those of LC-MS.

GC-MS

Suchy and Berry (2012) analysed by GC-MS the SPME fibres after Lemieux oxidation and methyl esterification of fish samples. Fragmentation of the MMPB methyl ester in electronic impact (EI)-MS does not present a molecular ion peak (M+ 222), but the fragments at m/z 190, 135, 131, 91 and 75. The GC-MS results corresponding to the total MCs were naturally much higher than those by ELISA related to free MCs.

Williams et al (1997) also analysed mussel extracts by GC-MS after Lemieux oxidation and monitored the fragment ions m/z 176 and 117 detected in EI.

Detection: biological methods

Immunological methods: ELISA

Berry and co-workers (2011) reported the use of an ELISA specific for the Adda moiety (Abraxis Kits); so did Preece et al. (2015). The polyclonal anti-Adda ELISA has good cross-reactivity with at least 80% of the known MCs because the antibody is raised against the unique C20 amino acid 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid (Adda) moiety found in all the MCs and NOD.

Another commercially available assay is the direct monoclonal (DM) ELISA which has very good cross-reactivity with all MC variants tested to date, including Adda (Preece et al., 2015). When MCs are

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detected by anti-Adda ELISA false positives can occur, due to cross reactivity with Nodularins; they also can give rise to an overestimation of the toxic potential of the biological sample due to cross reactivity with the GSH-conjugates, that have a much lowertoxicity. DM ELISA significantly reduced false positives compared with anti-Adda ELISA.

Tippkötter et al. (2009) developed an immunochromatographic lateral flow dipstick assay for the fast detection of MC-LR thanks to an anti-MC-LR Ab. Colloid gold particles with diameters of 40 nm were used as red-colored antibody labels for the visual detection of the antigen. Detection limit of 90 µg/kg of mussel flesh was achieved.

Enzymatic methods: PP inhibition assay

The protein phosphatase (PP) inhibition assay is based on the ability of MCs to inhibit Serine/Threonine protein phosphatase.

Williams et al. (1997) used a PP1c assay on mussel extracts in a ^{32}P-radiolabelled phosphorylase a phosphatase (PPase) bioassay to determine the equivalent unit quantity of microcystins in the samples.

The inhibition of Ser/Thr protein phosphatase by MCs in particulate and fish extracts was evaluated by a fluorometric method as described by Berry et al. (2011). Fluorescence of the product 4-methylumbelliferone was measured after 60 min incubation, using a Biotek Synergy HT microplate reader (λ ex = 360 nm; λ em = 460 nm).

The CIPPIA already presented for NOD (refer to section 4.2.2.3) has also been used for MC detection. In contrary to a classical PP inhibition assay, the CIPPIA specifically targets the inhibitory activity MCs and NOD towards PP and is not prone to interferences from other PP inhibitors such as OA, calyculin A, and tautomycin (Metcalf et al., 2001).

3.2.2. Nodularins

Sample preparation

Fish and shellfish: Extraction and cleanup

Sipia et al. (2001) extracted NOD from freeze-dried flounder *Platichthys flesus* and cod *Gadus morhua*livers by sonication with a mixture of water/MeOH/n- Butanol (BuOH) (75:20:5; v/v/v). The extracts destined to LC-UV analysis were further purified on C18 while those for ELISA or PP1 inhibition assay were left as is and were just diluted in water.

Konkaanpää and co-workers (2002) tested four different solvents on mussels *M. edulis*, clams *Macoma balthica* and flounders *Platichthys flesus*, for the extraction of NOD: (i) water/MeOH/n- BuOH (75:20: 5; v/v/v), (ii) water/MeOH/n-BuOH (65:30:5; v/v/v), (iii) ACN/0.1 M potassium dihydrogen phosphate (apparent pH 4.8; 15:85; v/v), and (iv) MeOH. The mixture of water/MeOH/n-BuOH (75:20: 5; v/v/v) was selected as the extraction solvent because less interferences were observed during analysis. This solvent mixture has been applied on different matrices such as flounder (*Platichthys flesus*) livers (Kankaanpää et al., 2005; Karlsson et al., 2003a; Vuorinen et al., 2009), blue mussel (*Mytilus edulis*) whole flesh (Karlsson et al., 2003a), flounder (*Platichthys flesus L.*), roach (*Rutilus rutilus L.*) and Eider (*Somateria mollissima*) liver and muscle samples (Sipia et al., 2006), three-spined stickleback (*Gasterosteus aculeatus L.*), herring (*Clupea harengus membras L.*), and salmon (*Salmo salar L.*) (Sipia et al., 2007).

Other extraction methods have been used to extract NOD from biological tissues, some of them using MeOH in different concentrations. Thus 75% aqueous MeOH has been tested to extract the toxin from freeze-dried blue mussel (*Mytilus edulis*) whole flesh, from flounder (*Platichthys flesus*) livers (Karlsson et al., 2003a), from liver of rainbow trout from aquaculture plants (*Oncorhynchus mykiss*) and liver from the common eider (*Somateria mollissima*) (Karlsson et al., 2005).

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A solution of 70% MeOH containing 1% trifluoroacetic acid has been applied on freeze-dried flounder livers prior to the analysis of the extracts by LC-MS or Maldi-TOF-MS (Karlsson et al., 2003b).
Tissues (liver, muscle) of mullet *Mugil cephalus* were extracted twice with 80% aqueous MeOH (Stewart et al., 2012).
Wood et al. (2012) extracted NOD from freshwater crayfish *Paranephrops planifrons* with 90% MeOH.
Prior to ELISA analysis, freeze-dried bile samples of flounder (*Platichthys flesus* L.) were extracted with 100% MeOH and diluted in water (Vuorinen et al., 2009).

Geis-Asteggiante et al. (2011b) extracted NOD from fish tissues (catfish, swai, and basa) with ACN/water (75/25) with 1% formic acid. The extracts were analysed as is by LC-MS/MS but for ELISA analysis, solvent was evaporated and formic acid in the extract was neutralised using a buffer (1 M Tris–HCl, pH 8.0). These authors also tested three other extraction procedures: (i) Quechers type, (ii) with ACN/Water (4:1, v/v), (iii) with acidified MeOH/Water (75:25, v/v) but they were not as efficient.

Prior to ELISA analysis, flounder (*Platichthys flesus*) and blue mussels (*Mytilus edulis*) tissues were disrupted in water, centrifuged and filtered. The mean recoveries were 90.7% and 86.9%, respectively (Zhou et al., 2011).

These extraction procedures were applied prior to analysis using different techniques, either chemical (e.g. LC-UV, LC-MS, Maldi-TOF-MS) or biological (e.g. ELISA, PP1, CIPPIA). For chemical analysis, the extracts were analysed with or without purification (liquid-liquid extraction, SPE); the extracts destined to biological methods were generally analysed without cleanup and after dilution to reduce the amount of organic solvent that would disturb the detection of the biological assays.

SPE is the cleanup procedure that has been used the most and applied to extracts obtained from various biological matrices. Reversed-phase cartridges have been largely used on extracts obtained from the mixture of water/MeOH/n-BuOH (75:20: 5; v/v/v) (Kankaanpää et al., 2005; Karlsson et al., 2003a; Konkaanpää et al., 2002; Sipia et al., 2001; Sipia et al., 2007; Vuorinen et al., 2009). Konkaanpää and co-workers (2002) tested 2 types of C18 cartridges (Bakerbond and Sep Pak).

Karlsson et al. (2005) also used reversed-phase (C18) SPE cleanup on a aqueous MeOH extract s of rainbow trout, mussel or common eider. The authors reported that matrix effects were observed by LC-MS/MS analysis when no SPE cleanup was applied: +30% for mussels, +10% for bird liver and -20% to -40% for fish liver. There were no matrix effects after SPE cleanup, except for fish liver with still ca. -40%.

Geis-Asteggiante et al. (2011b) used liquid–liquid partitioning with hexane to reduce the lipid content in the extracts obtained from fish tissues extracted with ACN/water (75/25) containing 1% formic acid.

Detection: chemico-analytical methods

LC-UV

For the analysis of NOD by LC-UV, the analytes are separated on a reversed-phase C18 column using mobile phases of different compositions: (A) 0.01 M $(NH4)_2HPO4$, pH 2.0 and (B) ACN (70.5:29.5, v/v) in isocratic mode (Konkaanpää et al., 2002; Sipia et al., 2001); (A) water and (B) ACN both with 0.05% FA with a gradient elution (Karlsson et al., 2003a).

The wavelength for the detection of NOD is 238 nm (Karlsson et al., 2003a; Konkaanpää et al., 2002; Sipia et al., 2001). Konkaanpää and co-workers (2002)reported that the LOQ ranged from 120 to 150 ng/g depending on the tissue analysed (refer to **Error! Not a valid bookmark self-reference.**.2.2.1). hese authors also reported that the toxin recovery was low (54-58%).

LC-MS/MS

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Similarly to LC-UV methods, separation prior to LC-MS/MS analysis was carried out on reversed-phase columns of different sizes (Geis-Asteggiante et al., 2011b; Kankaanpää et al., 2005; Karlsson et al., 2003a; Karlsson et al., 2003b; Karlsson et al., 2005; Sipia et al., 2007; Sipia et al., 2006; Stewart et al., 2012).

The mobiles phases are generally composed of (A) water acidified with 0.1% or 0.5% formic acid and (B) ACN (Kankaanpää et al., 2005; Karlsson et al., 2003a; Karlsson et al., 2003b; Karlsson et al., 2005; Sipia et al., 2007; Sipia et al., 2006); (A) water and (B) ACN both containing 0.1% formic acid (Geis-Asteggiante et al., 2011b).Other mobile phases have also been used: 10% ACN and 90% ACN, both containing 50 mM of formic acid and 2 mM of ammonia (Wood et al., 2012); (A) 1% aqueous MeOH, (B) 95% aqueous MeOH, both containing 0.1% formic acid (Stewart et al., 2012).
Whatever the mobile phases used, the analytes are separated on the columns in gradient mode.

Different types of mass spectrometers have been used: triple quadrupole (Geis-Asteggiante et al., 2011b; Kankaanpää et al., 2005; Karlsson et al., 2003a; Karlsson et al., 2003b; Karlsson et al., 2005), ion trap (Karlsson et al., 2003a), Maldi-TOF-MS (Karlsson et al., 2003b).

The detection was in positive ionization mode with the precursor ions m/z 825.5 and m/z 811.5 chosen for NOD and desmethyl NOD respectively, while the fragment ion m/z 135.1 was monitored for both toxins (Karlsson et al., 2003a; Karlsson et al., 2003b; Karlsson et al., 2005; Wood et al., 2012). Stewart et al. (2012)monitored the NOD precursor ion m/z 825.5 $(M + H)^+$ and the fragment ions m/z 135 and m/z 163. NOD glutathione (GSH) conjugate was monitored at m/z 1132.5 (Karlsson et al., 2003b). The cysteine (CYS) conjugates (m/z 946.5) can also be detected along with the NOD-GSH conjugates (m/z 1132.5) but the analytical conditions used by Karlsson and co-workers (2005) did not enable the separation of the 4 isomers of both NOD-CYS and NOD-GSH conjugate.
Geis-Asteggiante et al. (2011b) carried out analyses in negative ionization mode and monitored the NOD precursor ion m/z 823.6.

Detection: biological methods

Immunological methods: ELISA

ELISA methods have been used for the analysis of NOD in various matrices. As NOD is similar to MC in structure, the ELISA plate kits generally have a good cross-reactivity with MCs and NOD; this has been reported for the EnviroGard™ MCs plate kit, for which the recovery of NOD standard dissolved in water was 84% (Sipia et al., 2001).

The ELISA plate kit EnviroLogix™ also shows good cross-reactivity between MCs and NOD (Kankaanpää et al., 2005; Karlsson et al., 2003a) and was used in many studies for the analysis of NOD (Kankaanpää et al., 2005; Karlsson et al., 2003a; Sipia et al., 2006; Vuorinen et al., 2009).
The EnviroLogix kit plate has a detection limit of 0.2 g/L for NOD (Kankaanpää et al., 2005) and also shows cross-reactivity for hepatotoxin conjugates, such as glutathione conjugates (Kankaanpää et al., 2005; Karlsson et al., 2003a).

Geis-Asteggiante et al. (2011b) used an ELISA test from Abraxis that uses an antibody directed against the unusual amino acid Adda common to NOD and MCs, while Zhou and co-workers (2011) developed a competitive ELISA that uses a monoclonal antibody specific to NOD with no cross-reactivity with other toxins (<0.4%).

ELISA and PP1 inhibition assays used in parallel by Sipia et al. (2001) were in agreement most of the time except for muscle tissues of fish. Discrepancies between the ELISA and LC-MS/MS results have been reported; thus Karlsson et al. (2003a) observed higher results with ELISA compared to LC-MS/MS although the same extraction procedure had been applied. The authors explained the differences observed by the fact that the ELISA extracts were not purified by SPE in contrary to those

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analysed by LC-MS/MS where toxin losses might have occurred. Furthermore ELISA can detect the GSH and CYS conjugates, which were not by LC-MS/MS.

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Table 3.2.2.1: Information regarding the methods used for the analysis of NOD in various matrices

					Precision (% RSD)			
Author	Matrix	Detection method	LOD	LOQ	Intraday	Interday	Recovery (R) / Accuracy (A)	Matrix effects
Geis-Asteggiante et al. 2011	Fish	LC-MS/MS	<10 ng/g	-	-	10-20%	94±10% (R)	-2±12%
Geis-Asteggiante et al. 2011	Fish	ELISA	1.7 ng/g	-	-	-	-	-
Karlsson et al. 2005	Fish (L), Mussel (WF), Bird (L)	LC-MS/MS	S: 4 pg ClE: 13 pg CrE: 16 pg	-	-	-	-	≈ +30% (M)* -20 to -40% (F)* ≈ +10% (B)*
Konkaanpää et al. 2002	Fish (L), Mussel (WF), Clam (WF)	LC-UV		F: 150 ng/g M: 140 ng/g C: 120 ng/g	-	-	F: 54% (R) M: 58% (R) C: -	-
Sipia et al. 2001	Fish (L)	PP1 assay		<10 ng/g	-	-	W: 63% (R) F: 32% (R)	-
Sipia et al. 2001	Fish (L)	ELISA		<10 ng/g	-	-	W: 84% (R) F: 29% (R)	-
Metcalf et al. 2001	Water	CIPPIA	10 nM	-	-	-	-	-
Zhou et al. 2011	Fish	ELISA	0.16 µg/L	-	-	-	F: 90.7% (R) M: 86.9% (R)	-

(*)matrix effects measured without cleanup procedure; L= liver; WF= whole flesh; F=fish; M=mussel; B=bird; C=clam; S=solvent; ClE= cleaned extract;CrE=crude extract; W=water

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Kankaanpää et al. (2005) also reported that ELISA results were much higher (up to 10 folds) than LC-MS because of different extraction procedures, matrix effects, CYS and GSH conjugates detected with ELISA but not monitored by LC-MS.
Sipia et al. (2006) reported lower results withELISA than by LC-MS/MS for the analysis of liver and muscle tissues of flounders, roach and eiders.

Enzymatic methods: Protein phosphatase inhibition assay (PPIA)

The principle of the PPIA is based on the ability of NOD and MCs to inhibit the protein phosphatases, but this test also detects other compounds having the same biological activity such as okadaïc acid. Sipia et al. (2001) used a PP1 inhibition assay with a catalytic subunit of recombinant protein phosphatase 1 from *Escherichia coli*. The assay was carried out at 37±1°C for 2 h. The absorbance was read at 405 nm. Recovery of NOD standard dissolved in water was 63%. This method only detects free NOD.
ELISA and PP1 inhibition assays used in parallel by Sipia et al. (2001) were in agreement most of the time except for muscle tissues of fish.

Colorimetric immune protein phosphatase inhibition assay (CIPPIA)

In contrary to a classical PP inhibition assay, the CIPPIA specifically targets the inhibitory activity MCs and NOD towards PP. The ability of microcystin-LR antiserum to protect PP1 from the inhibitory action of microcystin-LR and related cyanobacterial toxins was used as the basis for the CIPPIA procedure (Metcalf et al., 2001). The assay uses the recombinant catalytic subunit of PP1 from rabbit skeletal muscle, expressed in *Escherichia coli*. Para-Nitrophenylphosphate is used as reagent turned into a coloured product, para-nitrophenol (p-NP) under the action of PP1. p-NP is detected at 410 nm. The specific action of MC and NOD is detected by comparing the result after preincubation with the preimmune serum or the MC-LR antiserum.
The LOD for NOD is 20 nM and 10 to 40 nM for MC variants (10 nM for MC-LR). CIPPIA is about 40 times more sensitive than HPLC with DAD; the detection limits, without sample concentration, were 10 and 400 µg/L, respectively (Metcalf et al., 2001).

3.2.3. Cylindrospermopsin

Sample preparation

Food supplements: extraction and cleanup

CYN can be extracted from BGAS using a 5.0% aqueous formic acid solution (Liu and Scott, 2011).

The crude extract is then purified on a combined "in-series" SPE system composed of a C18 column (500 mg/3 mL, Supelco LC-18) connected to a porous graphite carbon (PGC) column (HyperSep PGC, 100 mg/1 mL). The eluate from the PGC cartridge is evaporated, concentrated and analysed by LC-UV (Liu and Scott, 2011).

Fish and shellfish: extraction and cleanup

Guzman-Guillen et al. (2015) optimized and developed a method for the analysis of CYN in fish tissues, typically the muscle and liver of the Tilapia *Oreochromis niloticus*. CYN was extracted from the tissues using a mixture of water and ACN (30:70, v/v), containing 0.5% TFA.CYN can also be extracted from shrimps and fish fillets (catfish, trout, salmon, char) using the AOAC official method 2005.06 (Lawrence et al., 2005) that was initially developed for saxitoxin and its derivatives. Briefly, CYN is extracted from the matrix using a 1% acetic acid solution and the extract is filtered through 0.22 µm pore-size filters. The extract can be analysed as is, without any SPE cleanup, using LC-UV or by ELISA after a 500-fold (or more) dilution with deionized water (Niedzwiadek et al., 2012).

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Table 3.2.3.1: Information regarding the methods used for the analysis of CYN in various matrices

					Precision (% RSD)			
Author	Matrix	Detection method	LOD	LOQ	Intraday	Interday	Recovery (R) / Accuracy (A)	Matrix effects
Liu et al. 2011	BGAS	LC-UV	16 µg/g	52 µg/g	-	-	70–90% (R)	-
Guzman-Guillen et al. 2015	Fish	LC-MS/MS	L: 0.07 µg/g M: 0.002 µg/g	L: 0.12µg/g M: 0.007 µg/g	-	-	L: 80%-110% (R) M: 94%-104% (R)	-
Niedzwiadek et al. 2012	Shrimps, fish	LC-UV	-	S, F: 2 µg/g	-	-	F: 67.8-89.3%	-
Niedzwiadek et al. 2012	Shrimps, fish	ELISA	-	S, F: 0.05 µg/g	-	-	S: 75.-99.0% F: 65.7-75.6%	-

L = liver; M = muscle; F=fish; S=shrimp

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The fish tissue extract obtained with the method of Guzman-Guillen (2015) is further purified on Bakerbond C18 cartridges (500 mg, 6 mL) and Bond Elute PGC cartridges (500 mg, 6 mL). These SPE steps are not "in-series" as for the analysis of BGAS (Liu and Scott, 2011). The eluate from the C18 cartridge is passed through the PGC column. CYN is eluted using MeOH with 0.1% TFA; the eluate is evaporated to dryness and re-suspended in water, filtered and analysed by LC-MS/MS.

Detection: chemico-analytical methods

CYN can be analysed using LC-UV (Liu and Scott, 2011; Niedzwiadek et al., 2012) or LC-MS/MS (Guzman-Guillen et al., 2015).

The analytes are separated on reversed-phase columns prior to the detection; the stationary phases used are either classical C18 reversed phase (Niedzwiadek et al., 2012) or reversed phases designed for separating hydrophilic and polar compounds when using highly (Zorbax Sb–Aq, Genesis® AQ) (Guzman-Guillen et al., 2015; Liu and Scott, 2011).

The mobile phases used with the classical C18 column are composed of water with ammonium formate and ACN. For the Zorbax Sb–Aq and the Genesis® AQ columns, MeOH is used as organic solvent instead of ACN. Both the water and the MeOH phases contain either TFA (Guzman-Guillen et al., 2015) or ammonium acetate (Liu and Scott, 2011).

The performance of the LC-UV and LC-MS/MS methods are summarized in Table 3.2.3.1.3.1. The LC-UV method used for the analysis of fish and shrimps was much less sensitive than LC-MS/MS, whatever the tissue analysed (liver or muscle).

Detection: biological methods

The details of the Abraxis ELISA test used by Niedzwiadek et al. (2012) are not reported in the paper but can be found at the following URL: http://www.abraxiskits.com/wp-content/uploads/2015/02/Cylindrospermopsin-Insert-R021215.pdf.

The ELISA test of Abraxis had a LOQ of 0.05 µg/g for fish fillets and shrimps; this is 40 times lower than that of LC-UV (Niedzwiadek et al., 2012).

3.2.4. Anatoxins

Sample preparation

Food supplements: extraction and cleanup

Food supplements can be extracted with a mixture of MeOH:0.2 M HCl (75:25, v/v) and diluted with potassium hydrogen phosphate buffer (pH 6) before SPE cleanup and analysis (Rawn et al., 2007).

Rellan et al. (2009) also used acidified MeOH (1% HCl, 1M) for the extraction of BGAS. The combined extracts were evaporated to dryness under a N2 stream and re-suspended in water adjusted to pH 7.

BGAS were cleaned by SPE prior to derivatization and subsequent analysis. The adsorbents reported in the literature are of different types: C18 reversed phase (Rawn et al., 2007) or weak cation exchange (Rellan et al., 2009). Rawn and co-workers (2007) reported average recoveries of 93% ± 25%, 89% ± 17%, and 77% ± 24%, for ANTX-a, dihydro-ANTX-a and epoxy-ANTX-a, respectively

Fish and shellfish: extraction and cleanup

The extraction of ANTX from mussels *Mytilus galloprovencialis* described by Osswald et al. (2008) was performed on freeze-dried and ground tissues, using acidified MeOH (1% HCl, 1M), following the same procedure as that described by Rellan et al. (2009) for BGAS.

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ANTXs were extracted from oysters and clams (homogenised shellfish meat) using pure MeOH and Acetonitrile (ACN) and subsequently diluted in water before SPE cleanup (Rubio et al., 2014).

Bogialli and co-workers (2006) extracted ANTX-a from trout, mullet, perch and goldfish samples using a stainless-steel column used as extraction cell. The fish tissues blended with sand were packed in the extraction cell and the toxin was extracted with water acidified to pH 2 (HCl) passed through the cell at a constant flow rate and the final pH adjusted to 3.1.

The extract obtained from mussels (Osswald et al., 2008) was purified on a WCX SPE cartridge using the same procedure as described by Rellan et al. (2009) for BGAS.

In the case of the oyster and clam extracts, the SPE cleanup relied on the use of reversed phase C18 cartridge and the toxins were eluted using 60% aqueous MeOH (Rubio et al., 2014).

In the case of fish matrices, Bogialli and co-workers (2006) did not apply any specific cleanup procedure; the authors simply filtered the extracts on 0.2 µm pore size cellulose filters. They reported 71% to 78% recovery for ANTX-a from fish tissues (with RSD not larger than 9%).

Derivatization

No derivatization was required for the analysis by LC-MS/MS of the fish extracts from Bogialli and co-workers (2006). Inversely, the analysis of *M. galloprovincialis* extracts by LC-FLD required a derivatization procedure using NBD-F (4-fluoro-7-nitro-2,1,3-benzoxadiazole) (Osswald et al., 2008). The procedure was the same as that described by Rellan et al. (2009).

Detection: chemico-analytical methods

Gas chromatography

Rellan et al (2009) analysed BGAS by GC-MS and electron impact ionisation; this required the introduction of a derivatization step using a mixture of pyridine/acetic anhydride to form the more volatile n-acetyl ANTX-a. The reported limits of detection (LOD) and quantification (LOQ) were 24 ng/g and 70 ng/g, respectively. These limits of determination were higher than those reported by the same authors for LC-FLD analysis of BGAS after NBD-F (4-fluoro-7-nitro-2,1,3-benzoxadiazole) derivatization: 3 ng/g and 10 ng/g, respectively.
Furthermore, GC-MS suffered from matrix effects as 13% ion suppression was observed when analysing BGAS (Rellan et al., 2009).

A derivatization procedure is not compulsory, as Araoz et al. (2005) analysed cyanobacteria samples by GC-MS and screened the presence of the underivatized form of ANTX-a, M^+ m/z 165 (electron impact) and MH^+ m/z 166 (chemical ionisation). The limits of determination were not reported in the paper.

Liquid chromatography

Different types of detectors can be coupled to LC to detect ANTXs; this includes ultraviolet detectors (UV), fluorescence detectors (FLD) or mass spectrometers (MS).

Derivatization procedures are reported in the literature prior to the analysis of ANTXs by LC coupled to FLD to make the toxins fluorescent. The derivatization agent used is NBD-F (Osswald et al., 2009; Osswald et al., 2008; Rawn et al., 2007; Rellan et al., 2009). The derivatized form of the toxins can be analysed by mass spectrometry by monitoring the corresponding mass (Rawn et al., 2007) but a preliminary derivatization step is not necessary.
ANTXs can be analysed by LC-UV without any derivatization step (Aráoz et al., 2005; Dimitrakopoulos et al., 2010; Harada et al., 1993).

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The separation of ANTXs is generally performed on reversed-phase columns (C18) with mobiles phases of different compositions with ACN or MeOH as solvent, in gradient or isocratic mode (Bogialli et al., 2006; Furey et al., 2005; Furey et al., 2003; Harada et al., 1993; Osswald et al., 2009; Osswald et al., 2008; Rawn et al., 2007; Rellan et al., 2009). As phenylalanine (Phe) has the same nominal mass as ANTX-a (isobaric compounds) the LC conditions must enable the separation of both compounds; this is particularly important in low resolution LC-MS analysis as the resolution is too low to differentiate both compounds. In high resolution, both compounds can be distinguished based on their mass (ANTX-a: 165.11536 Da; Phe: 165.07898 Da) (Furey et al., 2005). Another approach consists in adding a methylation step in sample preparation using diazomethane prior to LC–MS determination as this removes Phe interference (Furey et al., 2005).

In UV, the monitored wavelength is 227 nm (Dimitrakopoulos et al., 2010; Harada et al., 1993) and corresponds to ANTX-a optimum absorbance whereas 250 nm would suit better for Phe (Dimitrakopoulos et al., 2010).
For FLD, the reported wavelengths for excitation and emission are respectively λ_{exc} = 470 nm and λ_{em} = 530 nm (Osswald et al., 2009; Rellan et al., 2009).
Several types of mass spectrometers have been used for the analysis of ANTX; simple quadrupoles (Harada et al., 1993), triple quadrupoles (Bogialli et al., 2006; Rawn et al., 2007), ion traps (Furey et al., 2005; Furey et al., 2003), hybrids quadrupole - time of flight (Furey et al., 2005) and orbitraps (Roy-Lachapelle et al., 2015).

Others

Roy-Lachapelle and co-workers (2015) developed a method for the analysis of water samples by laser diode thermal desorption–atmospheric pressure chemical ionization (LDTD–APCI) coupled to a high resolution/accurate mass spectrometer (HRMS). This method does not require any sample preparation (no extraction or purification) and due to the high resolution of the mass spectrometer, it distinguishes ANTX-a from Phe.

Nuclear magnetic resonance (NMR) has also been used for the quantitative analysis of ANTX (Dagnino and Schripsema, 2005).

Detection: biological methods

ANTX can be detected in environmental samples with biological methods such as an acetylcholine (ACh) esterase inhibition assay (Devic et al., 2002) or a ligand-binding assay using membranes purified from Torpedo electric organ rich in nicotinic ACh receptors [nAChR] (Aráoz et al., 2005). In the latter assay, ANTX-a and homo-ANTX-a inhibit ^{125}I-bungarotoxin binding at concentrations about two orders of magnitude lower than that of nicotine. Furthermore, both cyanobacterial toxins are equally potent agonists for nAChRs.
As for the AChE inhibition assay, the LOQ reported by the authors for the analysis of water samples is 0.5 nM.

3.2.5. Saxitoxins

Sample preparation

Shellfish: Extraction and cleanup

The extraction protocol described in the AOAC Official Method 2005.06 (Lawrence et al., 2005) has been used to extract STXs from various matrices: clams *Venerupis pullastra*, *Meretryx lyrata* and *Spisula solida*; mussel *Mytilus galloprovincialis* (Ben-Gigirey et al., 2012); mussels *Mytilus galloprovincialis* (Botelho et al., 2010); mussels (*Mytilus* sp.), Pacific oysters (*Crassostrea gigas*), native oysters (*Ostrea edulis*), cockles (*Cerastoderma edule*), king scallops (*Pecten maximus)*, queen scallops (*Aequipecten opercularis)*, surf clams (*Spisula solida*), and razor clams (*Ensis* sp.) (Turner et al., 2014).

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Briefly, STXs are extracted from the matrices with 1% acetic acid solution in a boiling water bath (100°C). Ben-Gigirey et al. (2012) introduced a hydrolysis step to convert GTX6 in NEO for which a standard is available. The fraction 2 collected after COOH SPE cleanup was hydrolysed with HCl, in a water bath, and the reaction was stopped with NaOH.

Other extraction methods are reported in the literature. Thus Laycock et al. (2010) and Watanabe et al. (2014) extracted STXs from mussels and the scallop *Patinopecten yessoensis*, respectively, according to the AOAC method 959.08. Briefly, the matrix is extracted with HCl 0.1M at 100°C.

Van de Riet and co-workers (2011) developed and validated a LC-FLD method, so called PCOX, for the analysis of STXs in blue mussels (*Mytilus edulis*), soft shell clams (*Mya arenaria*), sea scallops (*Placopectin magellanicus*), and American oysters (*Crassostrea virginicus*), after extraction of the toxins with 0.1 M HCl in a boiling water bath (100°C). A deproteination of the extract is performed using trichloroacetic acid and NaOH.

Rawn et al. (2009) extracted STXs from shellfish using 90% EtOH; the extracts were further diluted in buffer before being analysed by surface plasmon resonance (SPR) and Devlin and co-workers (2011) using PBS buffer (pH 7.2) to extract the toxins from mussels prior to cleanup using monoclonal antibody (mAb)-bound microspheres.

As part of the AOAC official method 2005.06, two consecutive SPE clean-ups are applied. The first requires the use of a C18 cartridge to purify the acidic extract obtained from the matrices. SPE-COOH ion exchange cleanup is used only for extracts that are found to contain N-1-hydroxylated PSP toxins after C18 cleanup. Three fractions are collected from the cartridge containing respectively the (i) C-toxins, (ii) the GTXs with B1 and B2 and (iii) NEO, STX, and dcSTX (Ben-Gigirey et al., 2012; Botelho et al., 2010; Turner et al., 2014).

Watanabe et al. (2014) also reported the use of SPE cleanup on a C18 cartridge for a scallop acidic extract prior to LC-FLD analysis.

Devlin and co-workers (2011) used a different cleanup approach. The STXs were trapped by a mAb raised against GTX 2,3 (GT-13A) and coupled to microspheres. The mAb-microsphere is reusable; a 92% recovery for STX in solution in PBS buffer, was reported after 35 usages of the microspheres. The recovery in mussel extracts showed a good recovery for STX (up to 84%), 62% recovery for GTX2,3 and less than 1% for NEO and GTX1,4 indicating a poor cross-reactivity.

Oxidation

The STXs have to be oxidised prior to LC-FLD analysis to make them fluorescent and detectable. As part of the AOAC Official Method 2005.06, the SPE-cleaned extracts go through periodate and peroxide oxidation procedures prior to the injection in the LC-FLD system (Ben-Gigirey et al., 2012; Botelho et al., 2010; Turner et al., 2014).

The oxidation protocol of the AOAC official method 2011.02 (van de Riet et al., 2011) is performed on-line, post-column, with an oxidizing agent (periodate) and stopped after the reaction coil within which the oxidation occurs, by adding nitric acid.

Watanabe et al. (2014) also used an on-line oxidation performed post-column, slightly different from that of van de Riet and co-workers (2011); the oxidizing agent was periodic acid and the reaction was stopped by adding acetic acid.

Detection: chemico-analytical methods

LC-FLD

Several authors reported the use of the so-called Lawrence method (AOAC Official method 2005.06)(Ben-Gigirey et al., 2012; Botelho et al., 2010; Devlin et al., 2011; Rawn et al., 2009; Turner

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et al., 2014). The toxins are separated on a reversed-phase column (C18) with a gradient of mobile phases (A: 0.1 M ammonium formate; B: 0.1M ammonium formate in 5% acetonitrile, both adjusted to pH 6) and detected by FLD (λ_{exc} = 340 nm, λ_{em} = 395 nm).
Ben-Gigirey et al. (2012) reported that the mean recoveries obtained for dc-GTX2,3 were, in general, low (53.1–58.6%). The RSD for reproducibility (RSDr%) for dc-GTX2,3 in all samples ranged from 28.2 to 45.7%, and HorRat values ranged from 1.5 to 2.8.
Mussel extracts exhibited a suppression of the fluorescence signal in comparison to solvent for dcGTX2,3, GTX2,3 and GTX1,4, while an enhancement was obtained for C1,2, STX, dcSTX, B1, dcNEO and NEO (Botelho et al., 2010), indicating that the use of matrix-matched standards is recommended for quantification.
Turner et al. (2014) adopted a semi-quantitative approach of the Lawrence method by performing periodate oxidation after C18 cleanup; this resulted in over-estimation of toxin levels by an average factor close to two in comparison with the fully quantified levels.

As part of the PCOX method (van de Riet et al., 2011), two types of chromatographic conditions are used for the separation/detection of STX and GTX toxins on one side and C toxins on the other side. Both sets of conditions require the use of reversed-phase columns although of different characteristics. The mobile used for STX/GTX is composed of heptane sulfonate against tetrabutyl ammonium phosphate for C toxins. Both mobile phases are run in gradient conditions. Detection wavelengths were set at 330 nm for excitation and 390 nm for emission.

In the LC-FLD method used by Watanabe and co-workers (2014), separation was achived on a reversed-phase column using a mobile phase (30 mM ammonium phosphate buffer containing 2mM heptane sulfonate pH 7.1/ACN ; 96/4 v/v) in isocratic conditions. Detection wavelengths were set at 330 nm for excitation and 390 nm for emission. This method enables the detection and quantification of STX using dcSTX as an external standard.

LC-MS

Poyer and co-workers (2015) developed a LC-MS method based on the principles of hydrophilic interaction liquid chromatography (HILIC) and ion mobility (IM) to separate and analyse the STXs. All the toxins are analysed in positive ionization mode but GTXs are also analysed in negative mode for a better sensitivity. HILIC-IM-MS is a complementary coupling that allows the differentiation of all the studied analogues; the separation of GTX isomers is achieved in the HILIC dimension while the non-sulphated analogues were separated in the IM-MS.

Detection: biological methods

Immunological methods

Kim and Choi (2015) developed a stationary liquid-phase lab-on-a-chip (SLP LOC) device, which is composed of a sample chamber connected to a detection chamber via a channel. The different reagents (buffer, anti-STX antibodies, STX-Horseradish peroxidase (HRP), and Protein G-coupled magnetic particles (G-MPs)) and the sample containing STXs are added to the sample chamber. A magnet was placed under the LOC and moved from the sample chamber to the detection chamber filled with HRP substrate solution (3,3′,5,5′-tetramethylbenzidine). The absorbance of the solution was determined at 650 nm with the plate reader.
The STX-HRP and STX present in the sample competitively bind to the anti-STX antibody attached to the G-MP, which is used as the solid phase. Therefore, the amount of STX-HRP bound MP is reciprocally proportional to the STX concentration of the sample. The LOD of the method is below 800 µg STX eq/kg.

The Jellet Rapid Testing kit for STXs is a competitive immunoassay in which toxins in the sample compete with toxin bound to the test line. In the absence of toxins in the sample solution, gold particles and their associated antibodies bind to toxin on the test line and form a visible red line

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(Laycock et al., 2010). The designed LOD of STX for the standard test is 250 µg STX 2HCl eq/kg. Some STX congeners have a poor cross-reactivity with the Ab (GTX1,4: 3%; NEO: 21%; dcSTX: 40%) making it difficult to detect them and thereby underestimating the toxin content.

Rawn et al. (2009) reported the use of the SPR methodology for the analysis of STXs. The detection limits (0.55-71.3 ng/mL) were higher than those determined by HPLC (0.16-1.29 ng/mL). Furthermore, SPR analyses generally resulted in higher STX levels relative to those obtained using HPLC and gave false positive and false negative results compared to HPLC.

Bioassays

Cladoceran cultures of *Daphnia pulex* Leydig and *Moina micrura* Kurs are used for the assay designed to detect immobilization of the cladocerans exposed to STX by preying on toxic cyanobacteria (Ferrão-Filho et al., 2010). The STXs paralyze the muscles of the second antennae, which is responsible for the swimming movements of the animals. The number of active swimming, immobilized and dead individuals is counted as part of the assay. The sensitivity level of the bioassay with *D. pulex* is in the range of 0.3 to 10.0 ng STX eq/L and in the range of 2.7 to 10.7 ng STX eq/L for *M. micrura*.

A speckled cockroach bioassay can be used to detect STX (Ruebhart et al., 2011). Animals of the species *Nauphoeta cinerea* Olivier were used for the bioassay. Prior to injection, the cockroaches were anesthetized by inducing a state of torpor (chill coma). The solution was then injected into the abdominal hemocoel of 10 animals. The endpoint assessed in the cockroach bioassay was "knock down" (KD) (animals unable to right themselves to a normal posture when inverted). The KD_{50} value, i.e. the dose affecting 50% of the tested animals, is 31.2 ng/g body weight at 60 min.

Cell assays

A cardiomyocyte-based impedance biosensor was developed by Wang et al. (2015); this assay uses the primary neonatal Sprague–Dawley (SD) rat cardiomyocyte and impedance sensor array, applied for monitoring cardiomyocytes beating status.
STX has no effect on beating amplitude of cardiomyocytes but inhibits the beating rates. STX showed time and concentration-dependent inhibition of beating rate. The reported LOD is 0.087 ng/ml for STX.

The basis of the receptor binding assay (RBA) is the competition between [^{3}H]STX and STX in a standard or sample for binding to the voltage dependent sodium channel present in rat brain membrane (Van Dolah et al., 2009).
The LOQ reported by the authors was 53 µg STX equivalents/kg of shellfish. The recovery from mussels spiked with STX averaged 121%. The two extraction methods used (0.1 M HCl and 1% acetic acid) showed linear correlation, with the HCl extraction method yielding slightly higher toxicity values. The RBA also had excellent linear correlation with HPLC analyses, but gave somewhat higher values than HPLC using either extraction method.

3.2.6. Lipopolysaccharides

Sample preparation

Lindsay et al. (2009) tested and compared four methods for the extraction of LPS from cyanobacteria (*Microcystis* sp., *Planktothrix* sp., *Aphanizomenon* sp. and *Cylindrospermopsis raciborskii*): the hot phenol/water extraction (HPW) (Westphal et al., 1952), two proteinase K extraction procedures (Eidhin and Mouton, 1993; Hitchcock and Brown, 1983) and the EDTA extraction protocol (Darveau and Hancock, 1983). The method that performed the best and yielded the highest LPS recovery is that of Eidhin et al. (1993), based on the use of Proteinase K and ribonuclease A for the extraction of the endotoxins.

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The HPW procedure is the most widely used for the extraction of LPS and has been applied to cyanobacteria samples (Bláhová et al., 2013; Keleti and Sykora, 1982; Mohamed, 2008; Papageorgiou et al., 2004; Weckesser et al., 1974). However, due to the toxic potential of phenol and the risks associated with its use in the HPW extraction procedure, other solvents mixtures have been used instead: hot butanol-water or hot N,N'-dimethylformamide –water (Mohamed, 2008; Papageorgiou et al., 2004). The extraction efficiency of these solvents mixtures was tested on different cyanobacterial strains and the efficiency was found to be dependent on the composition of the cell membrane (Papageorgiou et al., 2004). These authors stated that a single method cannot be regarded as universal and superior for the extraction of endotoxins from cyanobacteria.

Detection: biological methods

Limulus amœbocyte lysate (LAL)

The method of choice used for the analysis of LPS is the *Limulus polyphemus* amoebocyte lysate assay (LAL). Different approaches can be used for the detection: the gel-clot principle, based on the ability of LPS to make the *Limulus* amœbocyte clot; or the chromogenic approach based on the activation by the LPS of a protease that catalyses the cleavage of a chromogenic substrate, resulting in a coloured product measured by UV or fluorescence.

The gel-clot principle was applied in a LAL gelation kit assay, as reported by Keleti and Sykora (1982) and by Mohamed (2008).

The chromogenic approach was used either with UV detection (Bláhová et al., 2013; Lindsay et al., 2009; Papageorgiou et al., 2004) of fluorescence, as part of the PyroGene rFC endotoxin detection system (Bláhová et al., 2013).

Mouse bioassay (MBA)

Keleti and Sykora (1982) reported the use of a MBA to evaluate the lethal effect of purified endotoxin by intraperitoneal injections into 20-g Swiss-Webster female mice. However it is highly unspecific and it is generally used for the other toxins.

3.2.7. BMAA and its isomers

Sample preparation

BMAA can be extracted from different matrices as free toxin or bound to proteins. Different protocols are used depending on the extraction strategy chosen. The free toxin can be extracted first from the matrix; then the remaining pellet is extracted with another extraction solution that hydrolyses the matrix, thus promoting the release of the protein-bound BMAA. This approach gives 2 separate fractions containing the free and bound BMAA, respectively. Alternatively, the total BMAA content (free and protein-bound) can be determined by hydrolysing the matrix straight away.

Food supplements: extraction and cleanup

There are accounts in the literature of initial extraction of *Spirulina* tablets or powder with aqueous MeOH (20 to 80% organic solvent) (Jiang et al., 2013; Lage et al., 2014), prior to a hydrolysis step to release the protein-bound BMAA, to have both the free and bound toxin fractions.
Most often, BMAA is be extracted from BGAS as free toxin with 0.1-0.3M trichloracetic acid (TCA) (Rosen and Hellenas, 2008; Scott et al., 2009) while the protein-bound fraction is extracted with 6M HCl at high temperature (ca. 110°C) and for 17-24h (Jiang et al., 2013; Lage et al., 2014; Rosen and Hellenas, 2008; Scott et al., 2009).

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Jiang et al. (2013) included a liquid-liquid partition in the sample preparation to trap the non-polar compounds in chloroform and remove them from the total BMAA fraction obtained from *Spirulina* tablets. The extracts were further purified on Isolute HCX columns, which are mixed-mode SPE cartridges.

In the paper of Scott et al. (2009), the fraction extracted from BGAS containing free BMAA was purified on Strata strong cation exchange (SCX) cartridge, while the fraction with bound BMAA was first passed onto a C18 cartridge and then purified on SCX cartridge.

Plant matrices: extraction and cleanup

BMAA can be extracted from cycad seeds using the same protocols as for BGAS; briefly, the free toxin is extracted using 0.1M TCA (Faassen et al., 2012; Rosen and Hellenas, 2008), while the protein-bound toxin is obtained after 6M HCl extraction of the pellet from which the free BMAA has already been extracted (Rosen and Hellenas, 2008). The total BMAA fraction can be extracted directly from the cycad seeds and leaves using 6M HCl (Christensen et al., 2012; Faassen et al., 2012).

McCarron and co-workers (2014) reported the extraction of total BMAA from cycad plants using 6mM HCl, a concentration 1000 times lower than the protocols mentioned above.

The extracts obtained from cycad seeds or leaves were not purified prior to their analysis.

Fish, shellfish and other aquatic organisms: extraction and cleanup

Free and bound BMAA can be successively extracted from fish tissues (walleye, sauger, whitefish, pickerel) using 0.1M TCA and 6M HCl, respectively (Scott et al., 2009).

Reveillon et al. (2014) reported the extraction from mussels (*Mytilus galloprovincialis*) and oysters (*Crassostrea gigas*) of 2 fractions, containing respectively the free and bound BMAA, using successively 0.1M TCA and 6M HCl.
Andrys et al. (2015) extracted total BMAA from mussels *Mytilus edulis* using 12M HCl. The same total fraction was extracted from cockles *Cerastoderma edule*(Lage et al., 2014) and oysters *Crassostrea virginica* (Christensen et al., 2012) using 6M HCl.

Christensen and co-workers (2012) reported the extraction of total BMAA from blue crab *Callinectes sapidus* and sea hare *Stylocheilus striatus* using 6M HCl.

The fraction containing free BMAA was purified on SCX cartridge, while the fraction with bound BMAA was first passed onto a C18 cartridge and then purified on SCX cartridge (Scott et al., 2009).

Two kinds of polymeric cation-exchange sorbents, Bond Elut® Plexa PCX and Oasis® MCX were tested by Réveillon and co-workers (2014) for the purification of mussel and oyster extracts containing either free or bound BMAA and better recoveries were obtained with Bond Elut® Plexa PCX.Andrys et al. (2015) purified mussel extracts on Isolute HCX-3 SPE columns prior to the analysis of total BMAA.

Detection: chemico-analytical methods

LC-FLD

A fluorescence detector coupled to LC can be used for the detection of BMAA, but the extracts have to be derivatized to make the toxin fluorescent. Two different derivatization procedures avec been reported; they rely on the use of different reagents, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (ACQ) (Faassen et al., 2012) or 9-Fluorenylmethyl chloroformate (FMOC) (Scott et al., 2009).

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The constituents of the extracts are separated on C18 columns using a gradient of mobile phases of different compositions depending on the studies. ACN and water are used as solvents but with different salts such as ammonium formate, sodium acetate or triethylamine (Faassen et al., 2012; Scott et al., 2009).

The LOD and LOQ reported for the analysis of cycad seeds after hydrolysis (total BMAA) were respectively of 40 and 120 µg/g using the method of Faassen et al. (2012) with an intraday and interday precision of 2.8 and 4.6% RSD, respectively.

For BGAS extracts containing free or bound BMAA, the LOD and LOQ reported by the authors (Scott et al., 2009) are respectively of 0.21 and 0.69 mg/g and the LOQ for fish tissues of 0.22 mg/g.
Mean recoveries for BGAS and fish extracts are in the range of 67 to 89% for free BMAA and 66 to 83% bound BMAA (after hydrolysis).

LC-MS/MS

LC-MS/MS analyses can be performed on derivatized (Andrýs et al., 2015; Christensen et al., 2012; Faassen et al., 2012; Jiang et al., 2013; Lage et al., 2014; McCarron et al., 2014) or underivatized extracts (Faassen et al., 2012; Reveillon et al., 2014; Rosen and Hellenas, 2008) obtained from different matrices (BGAS, cycad plants, shellfish…). The derivatization procedures rely on the use of either ACQ (Andrýs et al., 2015; Christensen et al., 2012; Faassen et al., 2012; Lage et al., 2014; McCarron et al., 2014) or N-hydroxysuccinimide ester of N-butylnicotinic acid (C_4-NA-NHS) (Andrýs et al., 2015) as derivatizing agent. The precursor ion monitored for the presence of BMAA in positive ionization mode is either m/z 119 for the underivatized form or m/z 221 (BMAA-C4-NA) and m/z 459 (BMAA-ACQ) for the derivatized form of the toxin.

Separation prior to LC-MS/MS analysis was carried out on columns of different nature: C18 (Andrýs et al., 2015; Christensen et al., 2012; Jiang et al., 2013; Lage et al., 2014; McCarron et al., 2014), ZIC HILIC (Faassen et al., 2012; Reveillon et al., 2014; Rosen and Hellenas, 2008), TSK gel amide-80 (McCarron et al., 2014), Zorbax Eclipse AAA (Faassen et al., 2012) and sizes.

For the separation on C18 columns, the organic solvent used was either MeOH or ACN in combination with water. The mobile phase(s) was also composed of acetic acid, formic acid, ammonium formate and/or ammonia (Andrýs et al., 2015; Christensen et al., 2012; Jiang et al., 2013; Lage et al., 2014; McCarron et al., 2014).

The mobile phases run on the ZIC HILIC columns were composed of water and ACN with formic acid in both phases. The separation was done in isocratic conditions (Rosen and Hellenas, 2008) or with a gradient of mobile phases (Faassen et al., 2012; Reveillon et al., 2014).

For the TSK gel amide-80 and the Zorbax Eclipse AAA column, separation was achieved using a gradient of mobile phases composed of ACN, water and formic acid (Faassen et al., 2012; McCarron et al., 2014).

The performance of the different methods used for the analysis of BMAA and its isomers are summarised in 3.2.6.1.

Nuclear magnetic resonance

Moura et al. (2009) developed and validated a method for the analysis of BMAA by ^{1}H NMR in environmental aqueous samples. The toxin was extracted from the water by passing the sample through a Bond Elute Certify SPE column.

The performances of the method are described in Table 3.2.7.1. Briefly, the LOD and LOQ are respectively 5 and 10 ng/mL and the recovery ranges from 98 to 108% depending on the concentration level.

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Table 3.2.7.1: Information regarding the methods used for the analysis of BMAA and its isomers in various matrices

					Precision (% RSD)			
Author	**Matrix**	**Detection method**	**LOD**	**LOQ**	**Intraday**	**Interday**	**Recovery (R) / Accuracy (A)**	**Matrix effects**
Andrys et al. 2015	Shellfish	LC-MS/MS	Better sensitivity with C4-NA-NHS derivatization compared to ACQ		-	-	-	-
Christensen et al. 2012	Invertebrates	LC-MS/MS	0.5 µg/g	1.7 µg/g	2.4%	-	92% (R)	≈ +10%
Faassen et al. 2012	Cycad seed	LC-FLD	40 µg/g	120 µg/g	-	-	70-90% (R)	-
Faassen et al. 2012	Cycad seed	LC-MS/MS + derivatization	1.0 µg/g * 10.0 µg/g **	1.0 µg/g * 10.0 µg/g **	3.0%	5.0%	-	-
Faassen et al. 2012	Cycad seed	HILIC-MS/MS - derivatization	0.4 µg/g * 1.6 µg/g **	0.4 µg/g * 1.6 µg/g **	0.7%	1.9%	-	-
Jiang et al. 2013	BGAS	LC-MS/MS	0.1 µg/g	0.1 µg/g	3-6%	1-6%	103-105% (A)	+18%
McCarron et al. 2014	BGAS	RPLC-MS/MS	80, 36 and 211 ng/g for BMAA, AEG and DAB	-	-	-	-	-
McCarron et al. 2014	BGAS	HILIC-MS/MS	93, 44 and 205 ng/g for BMAA, AEG and DAB	-	-	-	-	-

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Table 3.2.7.1 (continued): Information regarding the methods used for the analysis of BMAA and its isomers in various matrices

					Precision (% RSD)			
Author	**Matrix**	**Detection method**	**LOD**	**LOQ**	**Intraday**	**Interday**	**Recovery (R) / Accuracy (A)**	**Matrix effects**
Moura et al. 2009	Water	^{1}H NMR	5 µg/mL	10 µg/mL	4.6-6.3%	6.1-8.4%	98-108% (R)	-
Réveillon et al. 2014	Shellfish	HILIC-MS/MS	-	0.15 µg/g	-	-	56-79% (R)* 64-81% (R)**	-6.3 to +7.3% -5.5 to +15.7%
Rosen et al. 2008	BGAS	HILIC-MS/MS	<1 µg/g * <4 µg/g **	-	-	-	-	-
Rosen et al. 2008	cyanobacteria	LC-MS/MS	-	-	2-6% * 1-6% **	-	95-104% (A)* 99-109% (A)**	-
Scott et al. 2009	BGAS	LC-FLD	0.21 µg/g	0.69 µg/g	-	-	67-89% (R)* 66-83% (R)**	-
Scott et al. 2009	Fish	LC-FLD	-	0.22 µg/g	-	-	67-89% (R)* 66-83% (R)**	-

(*) non hydrolysed extract (free BMAA); (**) hydrolysed extract (bound or total BMAA)

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3.2.8. Ciguatoxins

Sample preparation

Extraction and cleanup

CTXs were extracted from monk seals blood samples using ACN at solvent-to-blood volumes of 3:1 to precipitate the proteins and the final toxin extract was in 100% MeOH (Bottein et al., 2011). Mak et al. (2013) also extracted CTXs from blood samples but from coral reef fishes, using either ACN or MeOH at solvent-to-blood volumes of 3:1 or 10:1 (v:v)

Bottein et al. (2011) also extracted CTXs from other monk seals tissues, typically the liver, the brain and muscles, using acetone.

Different sets of conditions were used for the extraction of CTXs from fish flesh. Some protocols include a heat treatment at 60-70°C of the matrix prior to the extraction (Caillaud et al., 2012; Lewis et al., 2009; Stewart et al., 2010; Wong et al., 2014).
Wu et al (2011) validated an accelerated solvent extraction (ASE) protocol applied to coral reef fishes to extract CTXs. The toxins were extracted with MeOH under pressurized and warm conditions (1,500 psi, 75°C) and the final extract was in 50% aqueous MeOH.
The solvents or mixtures of solvents used for the extraction of CTXs from fish flesh are acetone (Abraham et al., 2012; Caillaud et al., 2012; Oshiro et al., 2010), MeOH (Epelboin et al., 2014) or MeOH : n-hexane(3:1, v/v) (Lewis et al., 2009; Stewart et al., 2010; Wong et al., 2014).

Freeze-dried pellets of *Trichodesmium* spp. were extracted with MeOH and partitioned with diethyl ether to look for the presence of CTX-like compounds using different methods (MBA, RBA, Neuro-2a) (Kerbrat et al., 2010).

Cleanup procedures were not necessarily required for the analysis of the studied matrices; two types of purification procedures were reported: liquid-liquid partitioning and SPE.

Liquid-liquid partitioning steps using n-hexane and diethyl ether were used to remove the lipophilic interferences and to extract the toxins of interest, respectively, from blood samples (Bottein et al., 2011) or fish extracts (Abraham et al., 2012; Caillaud et al., 2012; Epelboin et al., 2014; Oshiro et al., 2010).

Regarding SPE cleanup, C18 and silica (Si) SPE cartridges were used consecutively or alone on extracts of different nature: monk seals livers (Bottein et al., 2011), fish flesh (Abraham et al., 2012; Lewis et al., 2009; Stewart et al., 2010; Wong et al., 2014; Wu et al., 2011) and blood samples from coral reef fishes (Mak et al., 2013).

Detection: chemico-analytical methods

LC-MS/MS is the method of choice used for the analysis of CTXs in different matrices: monk seals (Bottein et al., 2011), fish (Abraham et al., 2012; Lewis et al., 2009; Mak et al., 2013; Oshiro et al., 2010; Wong et al., 2014; Wu et al., 2011).

The analytes are separated on reversed-phase columns (C6, C8, C18) of different characteristics with mobile phases composed of water, ACN, formic acid and sometimes ammonium formate and run in gradient mode. Oshiro et al (2010) used a mobile phase composed of water, MeOH and formic acid run in isocratic mode.

The CTXs were detected in positive ionisation mode. The precursor ion was either the MH^+ form or a $NH4^+$ or Na^+ adduct, the latter being less often reported. The fragment ions commonly monitored correspond to successive losses of water molecules. The adduct can also be lost in the fragmentation process.

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Detection: biological methods

Immunological methods

Zhang et al. (2015) developed an immunoassay with horseradish peroxidase and antibody-labelled gold nanoparticles probe hyphenated to capillary electrophoresis (CE) with electrochemical (EC) detection. This technique is a hybrid chemical and biological method. The detection limit for CTX-1B using the CE-EC immunoassay (0.045 ng/mL) was much lower than that of LC-MS used for comparison (1.72 ng/mL). Good recoveries (89.4-107.5%) were reported for the analysis of fish samples spiked with CTX-1B.

Tsumuraya et al. (2010; 2014) developed a sandwich ELISA using two anti-CTX mAbs prepared from synthetic haptens instead of natural toxins. These mAbs specifically bind to one of the two wings of CTXs. Depending on the mABs used, this ELISA detected 51-hydroxyCTX3C, CTX1B and CTX3C in a dose-dependent manner at less than 0.01, 0.28 and 0.8 ng/mL, respectively. No cross-reactivity was observed with other toxins such as brevetoxins, maitotoxin and okadaic acid.

Bioassays

The mouse bioassay (MBA) was used for the analysis of fish flesh (Epelboin et al., 2014; Oshiro et al., 2010) and *Trichodesmium* spp. cells (Kerbrat et al., 2010). Discrepancies were observed between MBA and LC-MS/MS results and could be explained by the fact that more CTX congeners were apprehended by the MBA and not by LC-MS/MS due to unavailability of standards (Oshiro et al., 2010).

Cytotoxicity assays

The Neuro-2a cytotoxicity assay was used to analyse monk seals tissues (Bottein et al., 2011), fish flesh (Abraham et al., 2012; Caillaud et al., 2012; Wu et al., 2011) and *Trichodesmium* spp. cells (Kerbrat et al., 2010). The Neuro-2a LOQ was estimated to be 0.32 ng/kg tissue equivalents (Bottein et al., 2011) and the cytotoxicity results correlated well with LC-MS/MS (Wu et al., 2011).
Another cell line can be used to detect CTXs that is the neuroblastoma x glioma hybrid cells NG108-15 (Cañete and Diogène, 2010). In the test conditions, an EC_{50} of 0.006 nM was reported for P-CTX-1 standard.

3.2.9. Palytoxins

Sample preparation

Extraction and cleanup

Palytoxins (PlTX) and analogues (ovatoxins [OVTX], ostreocin-D [OST-D]) were extracted from a wide variety of marine organisms: fish, molluscs, echinoderms, gastropods, crustaceans, cephalopods (Amzil et al., 2012; Biré et al., 2013; Brissard et al., 2014; Ciminiello et al., 2011; Suzuki et al., 2013).

The toxins were extracted from the matrices with MeOH in varying concentrations ranging from 50% (water:MeOH; 1:1, v/v) to 100% (Amzil et al., 2012; Biré et al., 2013; Brissard et al., 2014; Ciminiello et al., 2015; Selwood et al., 2012; Suzuki et al., 2013). Amzil et al. (2012) tested different MeOH concentrations and reported that 90% aqueous MeOH gave the best recovery. Ciminiello et al. (2011) also tested different extraction solvents, including acetone and ACN, and concluded that 80% aqueous MeOH gave the best results both in terms of average recovery and matrix effect.

Liquid-liquid partitioning steps have been reported as a cleanup procedure in the sample preparation of PlTX-contaminated extracts. The solvents used are dichloromethane (Ciminiello et al., 2011), hexane and chloroform (Suzuki et al., 2013).

SPE has also been used as a cleanup step prior to the analysis of the extracts. Different types of SPE cartridges have been tested: reversed-phase cartridges (Strata-X (Biré et al., 2013; Ciminiello et al.,

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2015; Selwood et al., 2012), Oasis HLB LP (Ciminiello et al., 2015)) and HILIC cartridges (PolyLC (Ciminiello et al., 2015)). Ciminiello et al. (2015) compared the different types of cartridges and identified Strata-X 500 mg as the best performing cartridge.

Selwood and co-workers (2012) included a micro-scale oxidation in the SPE procedure. The oxidation procedure is performed by passing periodic acid through the SPE cartridge while the toxins are trapped; the oxidation products are then eluted from the cartridge and analysed by LC-MS/MS.

Detection: Chemical methods

LC-MS/MS has been used for the analysis of PlTX and analogues in a wide variety of marine organisms including fish, molluscs, echinoderms, gastropods, crustaceans, cephalopods.

The analytes are most often separated on reversed-phase columns of different characteristics but Rossi et al. (2010) used a HILIC column instead. The mobile phases are composed of water and ACN, acidified with formic acid or acetic acid and sometimes also contain ammonium formate. These mobile phases are run in gradient.

The detection in MS/MS is in positive ionisation mode. PlTX and analogues can be multi-charged, therefore the precursor ion monitored can be of different forms: $[M+H]^{+}$, $[M+2H]^{2+}$ or $[M+3H]^{3+}$. Ciminiello et al. (2015) reported that in the analytical conditions of their study, the calcium adduct $[M+H+Ca]^{3+}$ was the most intense precursor ion.

Detection: Biological methods

Immunological methods

Boscolo et al. (2013) developed an indirect sandwich ELISA for PlTX analysis. The Abs used as part of the ELISA method were a mAb-PlTX as capturing reagent (immobilized into the wells of the 96-well plate) and an anti-PlTX polyclonal antibody (pAb-PlTX) used as a detecting reagent. A HRP-conjugated pAb directed against the pAb-PlTX is used. The presence of PlTX in the extract was evaluated by monitoring the coloured product formed under the action of HRP on 3,3,5,5-tetramethylbenzidine liquid substrate. The absorbance was read at 450 nm.
This sandwich ELISA has a LOQ of 11 µg PlTX/kg shellfish meat and does not cross-react with other toxins such as okadaic acid, domoic acid, saxitoxin, brevetoxin-3, and yessotoxin.

Cytotoxicity assays

The Neuro-2a assay can be used for the analysis of PlTX-contaminated samples (Ledreux et al., 2009). The specificity of the Neuro-2a cell-based assay for palytoxin detection was confirmed by using ouabain, which also acts on Na^{+}, K^{+}ATPase. The analysis of mussel extracts spiked with PlTX gave results correlated with those of the standard, although the EC_{50} was higher in mussel extracts.

The haemolytic potential of PlTX on red blood cells has been exploited as a basis for a haemolytic test. The use of mouse and sheep erythrocytes as part of the cytotoxicity assay has been reported by Biré et al. (2013) and Brissard et al. (2014), respectively. The haemolytic assay is more sensitive than LC-MS/MS with a LOQ of 1.2 µg PlTX eq./kg (Biré et al., 2013), and similarly to the Neuro-2a assay, ouabain was used to confirm the presence of PlTX and thereby rule out the fact that the haemolytic activity is due to other compounds. This assay was applied to a wide variety of marine organisms and there was a good agreement with the results obtained by LC-MS/MS (Biré et al., 2013).

Another approach is that of Volpe and co-workers (2014) who developed an electrochemical sensor for PlTX based on the measurement of the release of the cytosolic lactate dehydrogenase as an indicator of the haemolytic potential of the toxin on sheep erythrocyte.

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Receptor binding assay

Alfonso et al. (2014) developed a SPR biosensor assay relying on the interaction of PlTX and ouabain with immobilized Na^+,K^+-ATPase from dog kidney. The kinetic equilibrium constant (K_D) determined for the PlTX- Na^+,K^+-ATPase binding was in the range of K_D for unions between active biological species.

3.2.10. Concluding remarks on method performance

The concept of method performance is crucial to produce reliable data and must be addressed when considering the use of these data as part of risk assessment process. The detection technique used is only the end of the analytical chain; sample treatment is equally if not more important as it will affect the final result.

The first part of sample treatment is the extraction. Ideally, the method used must enable the quantitative extraction of the compounds of interest while discarding potential interferences. This proves to be very difficult to achieve, especially when dealing with complex matrices such as BGAS, plants and aquatic organisms (fish, molluscs, crustaceans etc.). Some toxins such as MCs and BMAA are known to exist as free toxins or bound to proteins; in such circumstances it is important to use the adequate extraction procedure depending on the objective that has been set (i.e. taking into account the free or total toxin in the study). The nature of the solvent mixture used as part of the extraction process must be chosen carefully to make sure of its compatibility with the detection technique; for instance, biological techniques are not compatible with high organic solvent concentrations.

The use of clean-up steps in the analysis of cyanotoxins has been largely reported and concerns different procedures such as liquid-liquid partitioning, SPE, SPME and IAC. If these clean-up steps aim at reducing the matrix load and thereby the interferences, they are also likely to reduce the toxin content through losses during the process. Unfortunately, the recovery rates of such procedures are not always reported when they should be if one wishes to correct the result for recovery, to have the most accurate estimate of the toxin content in the analysed matrix.

Regarding the detection techniques used for the analysis of cyanotoxins, they can be classified as chemical (CE, GC or LC coupled with UV, FLD or MS) or biological (bioassays, immunoassays, cytotoxicity assays, receptor binding assays). The performance of these techniques is assessed via characterization and/or validation studies. The criteria of interest are as follows: limits of detection and quantification, linearity, range, specificity, accuracy, precision (repeatability and reproducibility [or intermediate precision]), ruggedness. They are not all relevant depending on the type of method used, i.e. quantitative or qualitative, and different national and international standards/guidelines are available and can be followed as part of a validation process. Full validation studies on cyanotoxins are scarce and there are even fewer records when only considering BGAS, plants and aquatic organisms (i.e. excluding water).

During the review process we came across articles related to chemical methods that underwent characterization/validation studies, LC-MS being frequently reported as detection method. Yet, this technique is prone to matrix effects, responsible for inaccuracy due to ion enhancement or suppression especially in ESI mode. One can regret that these matrix effects are not systematically assessed. If LC-MS is very specific, especially in high resolution, this also constitutes one of its drawbacks. Indeed, this technique is used for targeted analyses, meaning that we can only detect what we are looking for. This applies to a lesser extent to the other chemical methods relying on UV or FLD as detection principle.

On the side of biological techniques, immunological methods (ELISA, SPR) are semiquantitative methods which can also be hampered by matrix effects. If the use of (monoclonal/polyclonal) antibodies directed against a toxin/toxin family helps recognizing analogues of the same family (without the need to have an analytical standard), the cross-reactivity of the antibody towards the toxin analogues can greatly vary among variants, making difficult any quantification. Other semi-quantitative methods such as cytotoxicity assays (haemolytic assay for PlTX, Neuro-2a for PlTX and

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STX, LAL test for LPS…) and receptor binding assays (PPIA for NOD and MCs, nicotinic AChR for ANTX…) have been reported for the analysis of cyanotoxins in complex food matrices with occasionally characterization data to assess their performance.

There are records in the literature about methods comparison but it can be difficult to come to a conclusion because of analytical bias such as the use of different extraction procedures. Furthermore, the observations made are very toxin/matrix dependent and may be different for another toxin/matrix couple.

Based on all these elements, when handling occurrence data as part of risk assessment, a higher weight should be given to those obtained with methods taking into account all the toxin fractions (free and bound), incorporating clean-up steps assessed for recovery and for which characterization/validation data are readily available for the considered toxin/matrix couple.

The provision of characterization/validation data should be encouraged as it would increase the level of confidence in the data produced and a requisite towards this objective would be the availability of standards and reference materials of the different toxins, which are lacking at the moment.

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3.3. Hazard identification and characterization

Individual data coming from the ELS are available in the inventory in Appendix C in tabular format, as MS Excel spreadsheets, which can be can be downloaded from the EFSA website in a Microsoft Excel format together with this report Data were divided in subsets, one for each cyanotoxin; in addition within each subset, distinct MS Excel spreadsheets were used for in vitro, in vivo and epidemiological studies.

3.3.1. Microcystins

Microcystins (MC) are a group of about 100 different variants (or congeners) of cyclic peptides consisting of seven amino acids. They are characterized by the presence of the amino acid Adda [(2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid], which is unique for cyanobacteria. Structural variations occur by the changing of two MC amino acid(s), and several other changes in small side groups. The chemical structure of MC-LR is shown in Figure 3.3.1.1.

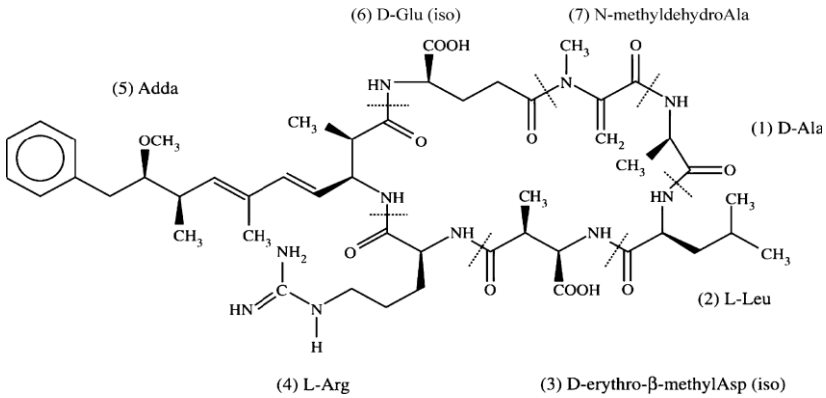

Figure 3.3.1.1: Chemical structure of MC-LR, with leucine (L) and arginine (R) as L-amino acids in positions 2 and 4

MC-LR is the most common and most studied MC congener, characterized by the presence of leucine (L) and arginine (R) as L-amino acids in positions 2 and 4 (Carmichael, 1988). On the basis of acute toxicity, MC-LR is considered amongst the most potent hepatotoxins within the different variants and is by far the most studied.

The number of studies retrieved was much higher than expected. Priority for data extraction, as described in the Methodology section, has been given to those *in vivo* studies useful for the derivation of health based reference values, which are unfortunately not many.

Amongst the retrieved papers, there are many *in vitro* studies and a large number of publications related to the cellular/molecular processes (Mechanism of Action (MeA)), particularly in relation to phosphatase inhibition, which are the base for the functional or physiological changes caused by the toxin (Mode of action (MoA)). For phosphatase inhibition, a number of publications are also available on fish.

The most recent studies are focused mainly on reproductive toxicity and organ toxicity other than liver (e.g. thyroid) and the use of OMICs technologies to investigate MoA and MeA, mainly on fish and generally using the i.p. route of exposure.

ADME

The accepted pathway for MC metabolism and excretion is GSH conjugation. Conjugates can be formed spontaneously at alkaline pH (Kondo et al.,1992) and enzymatically, catalysed by GSTs (Kondo et al., 1992; Pflugmacher et al., 1998). The GSH conjugate formation, mainly with MC-LR, has been

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reported *in vivo* and *in vitro* (Kondo et al.,1996; Takenaka 2001; Pflugmacher et al., 2005; Huang et al., 2008; Šetlíková and Wiegand 2009; Wu et al., 2010; He et al., 2012). The GSH adducts are much less acutely toxic than the parent compound (Botha et al.,2004); and are mainly excreted in the urine (Dittmann and Wiegand, 2006).
Recently GSH conjugation of MC-LR and MC-RR has been characterized using recombinant human GST and human liver cytosol from different pools of donors (Buratti et al., 2011, 2013, Buratti and Testai, 2015). The reaction involves the methyl group of *N*-methyldihydroalanine (opposed to Adda).

The uptake, tissue distribution, and excretion result in three- to four-fold differences among some congeners (Meriluoto et al., 1990). Since the hepatic organic anion transporters OATP1B1 and 1B3 are differently active in the MCs' entrance into primary human hepatocytes (Fischer et al., 2010), and also MC biotransformation efficiency is dependent on variant, with a higher rate of conjugation for MC-RR, the less toxic congener (Buratti and Testai, 2015), kinetics can be one of the reason for the congener-dependent toxicity.
For the moment very few information are available on variants other than MC-LR, especially for the more lipophilic ones for which kinetic parameters can be significantly different.

After i.p. injection of a dose of 70 µg/kg in mice, liver weight was reported to double after 60 and 90 min. From 3 to 90 min the radiolabel was detected in the kidney (0.92%), small intestine (6.4%) and large intestine (2.1%). There was a linear accumulation in the liver for 30 min (1.6%/min) and a corresponding decrease in the carcass. From 3 to 30 min, the plasma levels correspond to 5.5-8% of the administered dose in plasma with an elimination half-life of 29 min (Robinson et al., 1989).

Data obtained after intravenous injection of 35 µg MC-LR/kg bw are shown below (Robinson et al., 1991).

t½	=	0.8	Min	alpha phase	Fasted animals
t½	=	6.9	Min	beta-phase	Fasted animals
AUC	=	1036	Picomole per min per mL		Fasted animals
Plasma Clearence	=	0.9	Milliliter per min		Fasted animals

Very little *in vivo* data are available to allow kinetic parameters to be derived and limited to MC-LR.

Although not analysed for data extraction, some *in vitro* data are available and summarised below.
MC-LR is highly hydrophilic and cannot enter cell membranes by passive transport. It is actively absorbed by the intestinal mucosa, thanks to the organic anion transport system (OATP) and then enters hepatocytes through to the activity of bile acid transporters and OATP. These features have been demonstrated *in vitro* (Eriksonn et al., 1990; Fisher et al., 2005; 2010). These active transporters are expressed also in the kidney and in the blood–brain barrier, partially explaining some neurological disorders observed in humans during a fatal incident in Brazil (Azevedo et al., 2002). The uptake of radiolabeled dihydro-MC-LR has been demonstrated to be rapid in fresh hepatocytes in suspension and in perfused liver at 37°C: hepatocyte necrosis was observed 45 min after initiation of perfusion (Hooser et al, 1991). About 65-77% of the radiolabel was in the cytosolic fraction (of which 60-80% was bound to protein); 13-18% was in the plasma membrane (Hooser et al, 1991).When isolated rat livers were perfused with 0.3 and 0.5 µg/mL of tritiated MC-LR for 60 min distribution of radiolabeled was: 1.7% to bile, 79% to perfusate (the great majority of which was associated to the parent toxin) and 16% to the liver, associated to the cytosolic fraction and corresponding to parent toxin (15%) and a more polar component (85%, likely the conjugation metabolites). The elimination half-life from perfusate was 130 ± 10 min (at 0.5 µg/mL)(Pace et al., 1991).

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Genotoxicity

In vitro, literature data indicates that MC-LR is not genotoxic in the Ames test (Sieroslawska, 2013; Ding et al, 1999). Some positive results could be attributed to secondary effects related to cytotoxicity or to the use of extracts which show a different genotoxic pattern compared with the pure toxin. In the SOS ChromoTest (1-10 µM), dose-dependent induction of positive response without metabolic activation was reported (Mankiewicz et al, 2002). In the UmuC test performed without metabolic activation, at the lowest concentration (0.5 µg/ml) cytotoxic and genotoxic effects were observed. However, the human relevance of these results is limited since the UmuC test is not a part of the validated tests for genotoxicity (Sieroslawska and Rymuszka, 2010).

In some mammalian cell lines, the micronucleus and comet assay evidenced DNA damage associated with aneugenic effects (Dias et al,2014), although MC-LR was not active in other assays (in many cases due to absence of active uptake by the cells). Again the cyanobacterial extracts were reported to give positive results, whereas the pure MC-LR was not genotoxic (Žegura et al 2008, Mankiewicz et al., 2002).

MC-LR (2 and 10 ng/ml) significantly enhanced 8-oxo-dG, a common biomarker of oxidative DNA damage in time- and dose-dependent manner *in vitro* in primary cultured hepatocytes and *in vivo* in rat liver cells: the effect is compatible with the oxidative stress induced by the MC-LR MoA, not implying a direct interaction with DNA. MC-LR significantly decreased the amount of endogenously formed DNA adducts, especially after prolonged exposure (Bouaïcha et al., 2005).

Significant increase in DNA damage of primary cultured rat hepatocytes treated with MC-RR (0.01, 0.1, 1 µg/ml) (compared to the (solvent) control) was observed only after prolonged treatment (13 h). With human Hep G2 cells similar results were obtained (Žegura et al., 2002).

In vivo data on MC-LR are mainly obtained after a single exposure with doses closer or in the range of the LD_{50}: although induction of DNA damage in the liver as well as in other organs (e.g. testes) were reported the relevance is very limited, due to the high dose tested, considering that necrotic cells always have damaged DNA. After oral administration at a relatively high dose (2 and 4 mg/kg bw), a statistically significant dose-dependent but transient DNA damage in the blood cells was observed after 3h (not persistent at 24 h) (Gaudin et al., 2008).
A one-month repeated exposure to a sub-lethal dose (10 µg/kg i.p.) of MC-YR showed a significant increase of DNA damage (% tail DNA) in rats, with variable distribution of lesions in liver, kidney medulla and cortex, lung and brain. No DNA damage was detected in spleen and lymphocytes. The highest level of DNA damage was induced in brain, followed by liver > kidney medulla> kidney cortex > lung (Filipič et al., 2007).
Some indications about the MeA for secondary genotoxicity can be derived from the abstracted records, as the induction of DNA breaks were associated to ROS formation, mutagenic oxidative DNA lesions, interference with DNA repair and clastogenic activity, but not to direct interaction. More paper specifically dealing with MeA and MoA are reported in the sub-group MoA of the Endnote database.

Acute toxicity

As it is illustrated in table 3.3.1.1, most of data available on the acute toxicity of MCs refer to MC-LR for which data are available after oral, i.p. or i.v. administration; only a few other variants have been tested, exclusively by using i.p. injection.

Acute toxicity of MC-LR after i.v., i.p. and oral administration to mice results in LD_{50}s value of 28 µg/kg bw (Kondo et al., 1992), LD_{50} = 30-150 µg/kg bw, LD_{50} = 5000 µg/kg bw respectively. These values are of similar value obtained in the rat.

Acute toxicity is highly variable among MC variants, with LD_{50}s spread in a wide range of values (from 50 up to 600 µg/kg). The change of a single amino-acid in MC-RR results in an almost 10-fold lower toxicity when compared to MC-LR.

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Table 3.3.1.1 Data on MC acute toxicity in mammals expressed as LD_{50} values.

Variant	LD_{50}	value	SD (±)/ 95%CI (range)	Unit	Species	References
MC-LR	LD_{50} oral (gavage)	>5		mg/kg bw	Rat	Fawell et al., 1999
MC-LR	LD_{50} oral (gavage)	**5-10.9** Overall range		mg/kg bw	Mouse	
		≈ 5		mg/kg bw		Fawell et al., 1999
		= 10.9		mg/kg bw		Yoshida et al., 1997
		= 9.625		mg/kg bw		Rao et al., 2005
MC-LR	LD_{50} i.p.	**72-122** Overall range		µg/kg bw	Rat	
		= 106		µg/kg bw		Moreno et al., 2005
		= 12 (fed rats)	106-141	µg/kg bw		Miura et al., 1991
		= 72 (fasted rats)	60-83	µg/kg bw		Miura et al., 1991
		= 82.7		µg/kg bw		Li et al., 2014
MC-LR	LD_{50} i.p.	**32.5-158** Overall range		µg/kg bw	Mouse	
		= 32.5	± 1.2	µg/kg bw		Lovell et al.,1989
		= 97		µg/kg bw		Stotts et al., 1993
		= 36		µg/kg bw		Stoner et al., 1989
		= 65	53-80	µg/kg bw		Robinson et al., 1989
		= 76	65-90	µg/kg bw		Robinson et al., 1989
		= 43	37.5-49.4	µg/kg bw		Gupta et al., 2003
		50-158 (LD_0-LD_{100})		µg/kg bw		Fawell et al., 1999
		= 50	±5	µg/kg bw		Chen et al., 2006

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Variant	LD_{50}	value	SD (±)/ 95%CI (range)	Unit	Species	References
		= 65.4		µg/kg bw		Yoshida et al., 1997
		= 43		µg/kg bw		Rao et al., 2005
		= 70		µg/kg bw		Wang et al., 2012
		= 127	99-164	µg/kg bw		Chernoff et al., 2002
MC-RR	LD_{50} i.p.	**111-650** Overall range		µg/kg bw	Mouse	
		≈ 650		µg/kg bw		Stotts et al., 1993
		= 111		µg/kg bw		Stoner et al., 1989
		= 235.4	202.3-272.8	µg/kg bw		Gupta et al., 2003
		= 320	±10	µg/kg bw		Chen et al., 2006
MC-YR	LD_{50} i.p.	≈ 171		µg/kg bw	Mouse	Stotts et al., 1993
		= 110.6	81.7-149.6	µg/kg bw		Gupta et al., 2003
MC-WR	LD_{50} i.p.	≈ 171		µg/kg bw	Mouse	Stotts et al., 1993
		=140	±20	µg/kg bw		Chen et al., 2006
MC-FR	LD_{50} i.p.	≈ 249		µg/kg bw	Mouse	Stotts et al., 1993
		= 100	±10	µg/kg bw		Chen et al., 2006
MC-AR	LD_{50} i.p.	≈ 249		µg/kg bw	Mouse	Stotts et al., 1993
MC-LA	LD_{50} i.p.	= 39		µg/kg bw	Mouse	Stoner et al., 1989
MC-LY	LD_{50} i.p.	= 91		µg/kg bw	Mouse	Stoner et al., 1989
[desmethyl^7]MC-LR ([Dha^7]MC-LR)	LD_{50} i.p.	≈ 250		µg/kg bw	Mouse	Stotts et al., 1993

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Variant	LD_{50}	value	SD (±)/ 95%CI (range)	Unit	Species	References
[O-demethyl-ADDA[5]]MC-LR	LD_{50} i.p.	≈ 97		µg/kg bw	Mouse	Stotts et al., 1993
[desmethyl[7]]MC-RR ([Dha[7]]MC-RR)	LD_{50} i.p.	= 420	±30	µg/kg bw	Mouse	Chen et al., 2006
		= 180	73-449	µg/kg bw		Kiviranta et al., 1992
[desmethyl[3]]MC-RR ([D-Asp[3]]MC-RR	LD_{50} i.p.	= 250		µg/kg bw	Mouse	Meriluoto et al., 1989
		= 350	±10	µg/kg bw		Chen et al., 2006
MC-[methionine-S-oxide]R	LD_{50} i.p.	≈ 750	Estimated dose	µg/kg bw	Mouse	Stotts et al., 1993
[desmethyl[3]]MC-FR ([D-Asp[3]]MC-FR)	LD_{50} i.p.	= 90	±10	µg/kg bw	Mouse	Chen et al., 2006
[desmethyl[3]]MC-WR ([D-Asp[3]]MC-WR)	LD_{50} i.p.	= 95	±10	µg/kg bw	Mouse	Chen et al., 2006
[3H]dihydroMC-LR (epimer 1)	LD_{50} i.p.	= 120		µg/kg bw	Mouse	Meriluoto et al., 1990
[3H]dihydroMC-LR (epimer 2)	LD_{50} i.p.	= 135		µg/kg bw	Mouse	Meriluoto et al., 1990
MC-LR and RR	LD_{50} i.v.	80		µg $MC\text{-}LR_{equiv}$ /kg bw	Rat	Wang et al., 2008
MC-LR	LD_{50} i.v.	28	±9	µg/kg bw	Mouse	Kondo et al., 1992
GSH-MC-LR (coniugate)	LD_{50} i.v.	630	±69	µg/kg bw	Mouse	Kondo et al., 1992
Cys-MC-LR (coniugate)	LD_{50} i.v.	267	±88	µg/kg bw	Mouse	Kondo et al., 1992
MC-YR	LD_{50} i.v.	91	±2	µg/kg bw	Mouse	Kondo et al., 1992
GSH-MC-YR (coniugate)	LD_{50} i.v.	304	±52	µg/kg bw	Mouse	Kondo et al., 1992
Cys-MC-YR (coniugate)	LD_{50} i.v.	217	±6	µg/kg bw	Mouse	Kondo et al., 1992

The MeA of MC is associated with specific inhibition of protein serine/threonine phosphatases (PP1 and PP2A, with the latter being inhibited at higher extent), altering phosphorilation of cellular proteins involved in signal transduction (Gehringer, 2004). A substantial number of papers have been retrieved dealing the protein serine/threonine phosphatases , in a number of species including fishes. Although the papers regarding the MoA were not analysed for data extraction, some information are reported below. Adda aminoacid stereochemistry and its double bonds configuration are crucial for MC-induced

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PP1 and PP2A inhibition, due to a covalent binding between a protein cysteine and the Adda-glu group (Harada et al., 1990). Adda is the unique non-proteinogenic amino acid found in cyanobacterial hepatotoxins, as microcystins and nodularins, which *per se* is not able to inhibit PP1 and PP2A, and it is not toxic when i.p. injected in mice even at very high doses (10 mg/kg bw) (Harada et al., 2004).

The inhibitory capacity of single MC congeners on PP1 and PP2A *in vitro* is comparable, with IC_{50} values in the nanomolar range (Hoeger et al., 2007; Monks et al., 2007; Fischer et al., 2010; Vesterkvist et al.,2012).
At levels of exposure > 32 µg/kg bw *in vivo*, MC-LR-induced inhibition of PP1 and PP2A produces a cascade of events (cytoskeleton alterations, lipid peroxidation, oxidative stress, apoptosis) leading to hepatic centro-lobular toxicity with intrahepatic hemorrhagic areas due to damage of sinusoidal capillaries (Gehringer, 2004). A large number of papers is available on these aspects of the MoA of MC-LR particularly for the induction of oxidative stress both *in vitro and in vivo* in different species. For example, in human cell lines MC-LR has been shown to induce oxidative stress and apoptosis.

MCs are generally considered hepatotoxic, although neurotoxic effects have been reported in *in vitro* systems for some specific MCs, such as MC-LF, MC-LW, and MC-LR (Feurstein et al., 2011; Li et al., 2012a,b).

Acute toxicity data for MC are also available for different fish species. Although not included in the inventory, the data indicate that as for mammals, the great majority of the studies were carried out with MC-LR by using intraperitoneal injections of extracted toxins. From the available data, species of fishes seem to be generally less susceptible to MC-LR toxicity compared with mammals, with LD_{50} values in different species and/or genera ranging from 50 to 400 µg/kg and 400 and 1000 µg/kg bw after oral and i.p. injection respectively.

Subacute/Subchronic toxicity

At *in vivo* doses of MC-LR below 20 µg/kg bw (typical of repeated long-term exposure), phosphatases inhibition induces cellular proliferation and hepatic hypertrophy (Gehringer, 2004) at variance with effects caused by higher doses s described above.
In **Table 3.3.1.2** the NOAEL that could be derived have been reported, divided according to the administration route. However, few studies have been conducted with pure MC-LR via the oral route, many have been conducted with cyanobacterial extracts. The key studies are indicated in Table 3.3.1.2.

Table 3.3.1.2 Data on MC-LR subacute/subchronic toxicity, with identification of a reference value.

Duration	Reference value	Value	Unit	Species/ Limitations/Note	Reference
Oral					
10 days (gavage)	NOAEL	= 600	µg/kg bw/day	**Mouse**. Reproductive toxicity study. No evidences of maternal toxicity (although the number of parameters checked for general toxicity are rather limited in this kind of studies)	Fawell et al., 1999
28 days (drinking water)	LOAEL	= 50	µg/kg bw/day	**Rat.** Although hystological hepatic damage indicated in the text (no picture nor detailed description) no alteration of enzymatic markers (ALT and AST) for hepatic damage observed.	Heinze, 1998

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Duration	Reference value	Value	Unit	Species/ Limitations/Note	Reference
13 weeks (gavage)	NOAEL	= 40	µg/kg bw/day	**Mouse.** 3 different doses tested.Slight hepatic damages were observed at the lowest-observed-effect level (LOAEL) of 200µg/kg bw/day in a limited number of treated animals, whereas at the highest dose tested (1 mg/kg bw/day) all the animals show hepatic lesions, consistent with the known action of MC-LR. **KEY Study**	Fawell et al., 1999
18 months (via drinking water)	NOAEL	≥ 20	µg/L	**Mouse.** A single dose is tested, no effects* seen at 20 µg/L MC-LR in drinking water. Unbound NOAEL. (*) sporadic alterations not associated with other treatment-related changes	Ueno et al., 1999
Intraperitoneal					
7 days	NOAEL	= 3.125	µg/kg bw/day	**Mouse**. The paper measured few blood parameters,no histopathology was performed. The NOAEL is derived based on the alterations of ALP and LDH at the immediately higher dose (6.250 µ/kg) which on the other hand were the only alterations observed at that dose.	Huang et al., 2013
7 days	NOAEL	= 3	µg/kg bw/day	**Mouse.** no positive control The NOAEL is derived considering DNA-protein cross-links and micronucleus test in mice testicle cells, the only checked parameter (uncertain reliability as a marker for adversity).	Dong et al., 2008
28 days	NOAEL	= 16	µg/kg bw/day	**Rat.** Limitation: low number of animals; use of an i.p. osmotic pump.	Guzman and Solter, 1999
28 days	NOAEL	= 3	µg/kg bw/day	**Rat.** Limitations: focus only on some hepatic parameters, use of an i.p. osmotic pump, use of only 5 animal per group, use of ELISA to detect MC in the tissue (possible matrix effect not considered at all).	Solter et al., 1998
30 days	LOAEL	= 0.5	µg/kg bw/day	**Mouse.** The paper only measured parameters related to normocytic anemia after i.p. injection (altered only at high doses): there are evenno data on mice weight.The LOAEL is derived on the basis of altered MN frequency at the lowest dose tested. However, there is no positive control, high apoptosis level possibly affecting results of MN induction.	Zhou et al., 2013
50 days	LOAEL	= 1	µg/kg bw/day	**Rat.** The paper focuses on the spleen impairment after i.p. injection therefore the LOAEL only refers to limited spleen related parameters. Spleen effects at the LOAEL very slight, although increased at the higher dose. Proteomic analysis of 48 protein was also available but not processed for data extraction.	Li et al., 2012c

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Duration	Reference value	Value	Unit	Species/ Limitations/Note	Reference
4 weeks	LOAEL	= 5	µg/kg bw/day	**Mouse.** The paper focuses on thyroid dysfunction after i.p. injection therefore the LOAEL only refers to limited thyroid related parameters. More general alterations, linked to metabolic disorders seen only at 20 µg/kg. Some mechanistic data available (protein and mRNA expression related to thyroid hormone receptors also available but not processed for data extraction).	Zhao et al., 2015
Inhalation					
7 days	NOAEL	= 3	µg/kg bw/day	Mouse. NOAEL derived on the basis of nasal/ respiratory tract lesions	Benson et al., 2005

Some data on repeated toxicity are also available in fish species, but these are beyond the object of the current report which focuses on human health. However, in the environment, fish are likely to be exposed to sublethal concentrations of microcystins over days or weeks during the blooming season, and therefore this kind of studies have a relevance for the environment protection (and only indirectly for human health, via accumulation of the toxins, i.e. the subject of the task on occurrence). Most of the studies were carried out dissolving the toxins (mainly MC-LR) in water: this route of administration was more representative of actual fish exposure with respect to i.p. (the preferred administration in acute toxicity studies). Several studies on different species and genera have evidenced severe organ damages following sub-chronic exposure to MCs, particularly in the liver, although most of them describe primarily qualitative results, i.e. pathological modifications or alteration of selected genes transcription profile, some alteration in hematological and biochemical parameters, or changes in enzyme activities. Therefore, there is no robust quantitative data allowing NOAEL derivation to draw conclusions on repeated toxicity in fish.

Chronic toxicity and Carcinogenicity

In vivo i.p. treatment for 15 weeks (once a week) with 10 µg MC-LR /kg bw did not induce hepatic tumors in transgenic mice (HBVgene x); only 1/26 wild-type animal had a basophilic adenoma (Lian et al., 2006).
After oral administration (by gavage) of 80 µg MC-LR/kg bw to mice 100 times in 28 weeks, no characteristic chronic injuries such as fibrous changes and nodule formation were observed (Ito et al., 1997). No tumors were evidenced dosing mice at 1mg/L in drinking water for 28 weeks; even using a tumor promoter, no difference was evidenced with respect to animals treated with the tumor promotor alone (Labine and Minuk, 2014).
Neoplastic hepatic nodules were observed in mice treated i.p. with MC-LR (20 µg/kg bw for 28 day) (Ito et al., 1997), as well as induction of aberrant crypt foci in colon after addition of MC-containing extracts in drinking water (Humpage et al., 2000).
These studies were not conducted according to any current guideline for carcinogenicity testing.

In the two-stage carcinogenesis model, the tumor-promoting activity of cyanobacterial extracts and pure MC-LR was demonstrated in some animal studies. Indeed, the tumor-promoting activity of MC-LR was described already early in cyanotoxin research (Nishiwaki-Matsushima et al., 1992), then confirmed by cyanotoxin administration with known carcinogenic compounds, such as aflatoxin B1 and diethyl-nitrosamine (Sekijima et al., 1999). Repeated MC-LR i.p. administration (10 µg/kg) induced the formation of GST-P(+) liver foci in the presence of an initiator (Sekijima et al., 1999). Generally the MC-LR tumor-promoting activity is associated with the inhibition of protein phosphatases; it has been suggested by Fujiki & Suganuma (2011), that the tumor promoting activity of MC-LR could involve TNFα, as an endogenous tumor promoter and a central mediator of tumor promotion.

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The International Agency for Research on Cancer (IARC) reviewed available data on MC-LR carcinogenicity, concluding that there is inadequate evidence in both humans and experimental animals for the carcinogenicity of MC-LR. However, IARC classified MC-LR as possibly carcinogenic to humans (Group 2B) on the basis of data on tumor promoting mechanisms (IARC, 2010).

Reproductive/developmental toxicity

Data of possible effects on reproductive and developmental toxicity are available: no teratogenic effects were observed but some limited evidences of effects on male reproductive systems have been reported (many of the papers originate from the same research laboratory and except in one case are associated to i.p. administration) Since those studies have weaknesses in experimental design and reporting they were considered not adequate for the derivation of a health-based value.
No teratogenic effect was observed when dams were treated orally up to 2000 µg/kg bw for 10 days, whilst maternal toxicity was evident. A NOAEL for reproductive toxicity of 600 µg/kg bw could be derived (no evidences of either maternal or foetal toxicity) (Fawell et al., 1999).
Cognitive effect on off-springs were observed on rat pups from dams treated orally with 1000, 5000, 20000 µg/kg bw for 8 weeks. Male and female pups showed altered abilities in the cliff avoidance test on Post Natal Day (PND) 7. Locomotor behavior was not affected on PND 28 and 60. In the Morris water maze test all groups exhibited decreased escape latencies. No evident pathological exposed alterations were observed in the CA1 regions of MC-LR-exposed rat brain (Li et al., 2015).
Also after i.p. injection no teratogenic effects were evidenced at dose levels up to and including 128 µg/kg bw for two days (Chernoff et al., 2002).

When mice were treated orally (in drinking water) for 3 and 6 months with MC-LR (commercial product; purity not stated) at concentrations of 0, 1, 3.2, or 10 µg/L (approximately 0, 0.25, 0.79 or 2.5 µg/kg bw/day using default data; body weight and amount of water consumed were measured, but these data were not presented and doses to the animals were not calculated by the authors), at doses ≥ 3.2 µg/L, a dose and time-dependent decline of sperm quality (↓motility and count, ↑ abnormalities) was observed associated with testicular atrophy (at 6 months). Dose-dependent statistically significant decrease of testosterone and increase of FSH and LH were also observed at 6 months starting from 3.2 µg/L. No clinical signs of toxicity were observed and body weight, testes weight, and water consumption were not affected by treatment. However, methodological and reporting limitations affect this study (e.g. use of methanol as a vehicle for MC-LR not administered to control animals, effects in the control group, poorly described analysis on sperm, no purity of the test item reported, nor check for MC-LR concentration in the administered drinking water) (Chen et al., 2011). Similar effects at similar range of doses (5- 15 µg/kg bw, purity not reported) were observed after 28 days i.p. treatment (Li et al., 2008).

Other studies are available in which MC-LR was administered i.p. to rodents: besides the poorly representative route of exposure, these studies are often affected by some limitations.

When rats were i.p. injected with 1 and 10 µg MC-LR/kg bw/day per 50 days, at the high dose some histological alterations were reported in the tests (no other parameters for general toxicity reported) as well as increased expression in some genes. FSH levels in the high dose group were significantly higher compared with the control group, and LH and testosterone levels were significantly increased and decreased (compared to controls) in both groups (Chen et al., 2013).

When mice were i.p. injected with 0, 3.75, 7.5, 15, or 30 µg/kg bw of MC-LR (purity not reported) for 1, 4, 7, or 14 days, animals in the 15 and 30 µg/kg groups had significantly lower body weights; as well as changes in gene expression corresponding to transient increases in FSH, LH, and testosterone levels at 7 days followed by decreases at all doses after 14 days of treatment (Wang et al., 2012).

In female mice treated i.p. for 28 days significant decrease of GSI, composition ratio of the number of primordial follicles, was observed at 20 µg/kg bw/d. Exposed mice exhibited an abnormal estrous

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cycle. Significant decrease of the duration of the pro-estrus and estrus stage observed at 20 µg/kg bw/d (purity not reported) (Wu et al., 2014).

Studies using non purified extracts containing MC-LR and –YR (LD_{50} of the test item: 35.20 and 33.3 µg MCs/kg bw) evidenced that when mice were i.p. exposed to 6 µg extracts/kg bw, there was a slight increase in the numbers of dead and resorbed embryos which reached the statistical significance at 12 µg extract/kg bw. No evident effects on skeletal development was reported in all the experimental groups, but 4 fetuses in the 6 µg extract/kg bw dose group were found to have a curving tail (Bu et al., 2006). In male mice no significant difference was found in the average concentration and abnormality of sperm, although statistically significant decrease in sperm viability and percent of rapid motility was reported (Ding et al., 2000).

Epidemiological data in humans

A number of epidemiological studies are focused on the description of the well-known poisoning episodes that occurred in Brazil. These episodes involved human intoxication to MCs through contaminated water used for dialysis purposes. The occurrence of the toxin was measured using ELISA assays, which can only detect free MC-LR, and not the protein-bound form. The results indicated average concentrations in human liver of 223 ng/g of MCs, namely for MC-YR, -LR, and -AR (the mean of the serum values was 2.2 ng/ml). Concentrations of unbound MCs in the water used for dialysis treatments were estimated to be as high as 19.5 µg/L MCs (Charmichael et al., 2001; Hilborn et al., 2013; Pouria et al., 1998).
Two ecological studies considered the possible correlation between incidences of primary liver cancer (Serbia) or primary hepatocellular carcinoma (Florida) and residence in locations where drinking water is known to be contaminated (Svirčev et al., 2009, 2013; Fleming et al., 2002). Although the studies could highlight some important geographical hot spot of exposure to MCs (particularly the Serbian study), the estimation of MC-exposure was concluded to have some limitations including the lack of information on the residency of patients in the 25 previous years considering the latency of the disease. Hence, these studies cannot be used as determinant for a causal relationship between MCs exposure and liver cancer.

Another study (from China) aimed to correlate incidence of colorectal carcinoma in relation to consumption of food and drinking water (from untreated ponds), contaminated with MCs. However, the presence of other contaminants or confounding factors (such as the endemic presence of viruses and AFB1) were not considered (Zhou et al., 2002).
A cohort study (on Chinese fishermen) and a cross sectional study (on Chinese children) were also available and consumption of contaminated water/food was correlated with the alteration of hepatic parameters (Chen et al., 2009; Li et al., 2011).
The cross sectional study had a large number of subjects (>1000), however, the subjects were exposed to a number of chemicals as well as other toxins and disease stressors (e.g. HBV infections, as confounding factors, AFB1 food contaminations and use of hepatotoxic drugs). AST and ALP were significantly increased in the high-exposed children (AST = 33.4 U/L, ALP = 288.7 U/L) compared with the low-exposed children (AST = 30.3 U/L, ALP = 285.6 U/L) and the unexposed children (AST = 27.6 U/L, ALP = 281.3 U/L). Test serum MC-LR concentrations showed the detection rates of 1.9%, 84.2%, and 91.9% in unexposed, low-exposed, and high-exposed children, respectively; the mean concentration in the high-exposed children was 1.3 µg MC-LReq/L higher than in the low-exposed children (0.4 µg MC-LReq/L) and unexposed children (< LOD). The risk factors for liver damage, as measured by serum enzyme levels, in relation with MC exposure showed an odd ratio (95% CI) of 1.72 (1.05– 2.76) (Li et al., 2011).
In the study on Chinese fishermen, the presence of MC-RR, MC-YR, and MC-LR in water was detected. In Lake Chaohu, in July, MC-RR, -YR, and -LR concentrations in seston were 3.74, 0.48, and 2.34 µg/l, respectively (in cyanobacterial scum were 351.6, 62.0, and 128.3µg/g, respectively). The toxin content was 3.28 µg MC-LReq/l in seston, and 0.223 mg MC-LReq/g (dry weight) in cyanobacterial scum. The mean MC-RR, -YR, and -LR concentrations in serum of the fishermen were 3.89, 1.24, and 4.20 ng/g dry weight (DW), respectively, the proportions of MC-RR, -YR, and -LR were 41.7, 13.3, and

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45.0%, respectively, close to the proportions of MC-RR, -YR, and -LR in the muscle of aquatic animals (42.1, 3.3, and 33.1 ng/g DW, respectively, in terms of MC-LReq, this equals to 42.8 ng/g DW). Absence of MC in 3 control samples. Totally, daily intake of MCs by a fisherman could be 2.2–3.9 µg MC-LReq. MC concentrations showed closer positive relationships with biochemical parameters ALT, AST, LDH, and ALP than with other biochemical indices (Chen et al., 2009).
In a Canadian case control study (prospective) involving people living close to a lake affected by MC-producing blooms, there was a significant increase in adjusted relative risk (RR) with higher cyanobacterial cell counts for gastrointestinal symptoms (<20,000 cells/mL: RR = 1.52, 95% CI = 0.65–3.51; 20,000–100,000 cells/mL: RR = 2.71, 95% CI = 1.02–7.16; >100,000 cells/mL: RR = 3.28, 95% CI = 1.69–6.37, p-trend = 0.001). In participants who received their drinking water supply from a plant whose source was contaminated by cyanobacteria, an increase in muscle pain (RR = 5.16; 95% CI = 2.93– 9.07) and gastrointestinal GI1 (diarrhea or abdominal pain or nausea or vomiting): RR = 3.87; 95% CI = 1.62–9.21; GI2 diarrhea or vomiting or [nausea and fever] or [abdominal cramps and fever]): RR = 2.84; 95% CI = 0.82–9.79), skin (RR = 2.65; 95% CI = 1.09–6.44) and ear symptoms (RR = 6.10; 95% CI = 2.48–15.03) was observed (Lavesque et al., 2014).

3.3.2. Nodularins

Nodularins (NOD) are cyclic peptides consisting of five (NOD) amino acids, including the characteristic amino acid Adda, which is a unique structural feature of toxins produced by cyanobacteria. Structural variations in the synthesis of the toxins occur through changing an amino acid at the time and these differences give rise to at least 6 NOD variants (Sivonen and Jones, 1999).
Nodularins share with MCs both a similar chemical structure and phosphatases inhibition as MeA (Yoshizawa et al., 1990). However, they have not been studied as extensively as MCs.

ADME

No data are available.

Genotoxicity

NOD has not been tested in bacteria. In an in vitro study with HepG2 cells NOD (1-10 µg/ml) increased the basal DNA strand breaks in dose-dependent manner at all times up to 24 h; however, at 48 h the overall level of damage was low. At 5 and 10 µg/ml for 24h NOD increased the relative frequencies of micronuclei containing centromeres (MNC+). A calculation of the absolute frequencies MNC+ and MNC- revealed that NOD only induced MNC+ (Lankoff et al., 2006).
NOD (2 and 10 ng/ml) significantly enhanced 8-oxo-dG, a common biomarker of oxidative DNA damage, in time- and dose-dependent manner in primary cultured hepatocytes and in vivo in rat liver cells. NOD significantly decreased the amount of endogenously formed DNA adducts, especially after prolonged exposure being more effective than equal dose MC-LR (Bouaïcha et al., 2005).

Acute toxicity

The i.p. LD_{50} in mice (50–70μg/kg bw) is similar to the one for MC-LR.

Subacute/subchronic toxicity

No data on short term repeated toxicity relevant for risk assessment are available. However, it is reasonable to consider that, as a worst case, NOD repeated toxicity can be compared with MC-LR and therefore to adopt the NOAEL value identified for MC-LR, expressing NOD concentrations as MC-LR equivalents.

Chronic toxicity and Carcinogenicity

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In an *in vivo* study NOD did not induce hepatic tumors in transgenic (HBVgene +) and wild-type mice treated (i.p.) for 15 weeks (once a week) with 10 µg NOD /kg bw (Lian et al., 2006).
Nodularins are considered tumor promoters without initiation capability (Ohta et al., 1994; Song et al., 1999). In the two-stage carcinogenesis models in rat, the tumor-promoting activity NOD (25 µg/kg bw i.p.) induced the formation of GST-P(+) liver foci in initiated animals with higher volume and area of foci/liver, but not number, than MC-LR. NOD alone induced hepatocyte proliferation until week 18 (↥ PCNA index) (Song et al., 1999).

However, according to the IARC evaluation, NODs are not classifiable as to their carcinogenicity to humans (Group 3), due to the scant amount of data available (IARC, 2010).

Reproductive/developmental toxicity

In a toxicity study testing the promoting activity of NOD, with the use of initiators, testosterone levels decreased after NOD injection (25 µg/kg bw for 2 days, i.p., no effect of initiator alone). 17α-hydroxylase and pregnolone levels were reported to decrease after NOD treatment with respect to control animals but the initiator alone showed the same effect at an even higher degree, being a relevant confounding factor. NOD alone induced loss of spermatogenesis, apoptotic changes in spermatogonia (pyknotic and shrunken), atrophic change in the prostate epithelium with apoptosis (nuclear dusts). Lysosomes induction in Sertoli cells in initiated and non-initiated rats, as well as increase of perixosomal granules in Leydig cells with signs of apoptosis were observed. Inhibition of expression of mRNA expression of stAR gene up to 80%, 60% and 30% in the initiator alone, plus NOD, and NOD alone rats, respectively, was evidenced (Park et al., 2002).

Epidemiological data in humans

No data are available.

3.3.3. Anatoxins

Anatoxin–a (ATX) is a secondary amine alkaloid with a molecular weight of 165, whose structure was defined by X-ray crystallographic analysis in 1977 (Devlin et al, 1977) (Fig. 3.3.3.1). It was first synthesized two years later (Campbell et al, 1979) as a mixture of stereoisomers (i.e. both optically positive and negative enantiomers).

Homoanatoxin-a (HTX) is a structurally related ATX analogue, with a molecular weight of 179 and different from ATX with a propionyl group at C-2 instead of the acetyl group (Fig. 3.3.3.1). It was first synthesised (Wonnacot et al, 1992) just before its isolation from a sample of *Oscillatoria formosa* (Skulberg et al, 1992). Due to its structural similarity to ATX, HTX is probably produced by the same biosynthetic pathway, with the additional carbon deriving from L-methionine via S-adenosyl-methionine (Namikoshi et al 2003).

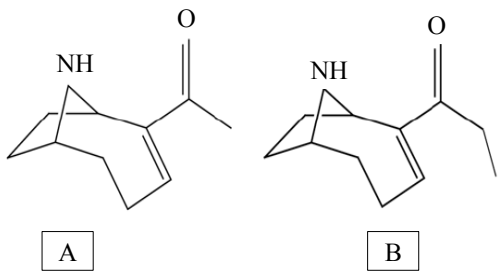

Figure 3.3.3.1. Chemical structure of (A) anatoxin-a and (B) homoanatoxin.

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Anatoxin-a(S) (ATX-s) despite the similar name has a completely different structure (Fig.3.3.3.2): it is an N-hydroxyguanidine methyl phospate ester with a molecular weight of 252. It is the only natural organophosphonate known. No structural variants of ATX-s have been detected so far.

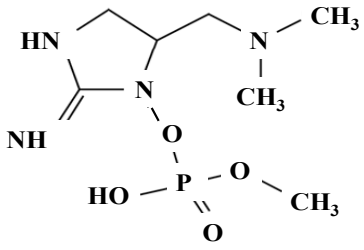

Figure 3.3.3.2. Chemical structure of anatoxin-a(s).

ADME

There are no *in vivo* data describing the kinetic behavior of anatoxins, from which it is possible to derive any parameters. However, considering the short lag-time between the administration of a lethal dose and death and their chemical structure it can be reasonably expected that anatoxins are rapidly absorbed and widely distributed to different tissues, including the brain; a low bioaccumulating potential can be anticipated.

Genotoxicity

Anatoxin–a and Homoanatoxin-a

No mutagenic activity in Ames test of pure ATX in a wide range of concentrations (0.312, 0.625, 1.25, 2.5, 5 and 10 µg/mL) has been found (Sieroslawska, 2013). In UmuC test without metabolic activation the first concentration to be no cytotoxic neither genotoxic was 0.25 µg/ml (Sieroslawska and Rymuszka, 2010).

No data are available on HTX.

Anatoxin-(a)S

ATX-S showed negative results when tested up to 2 µg/mL in the Ames test with or without metabolic activation, whereas with human lymphocytes *in vitro* an increase in chromosomal breakages was observed at the highest dose-level (0.8 µg/mL): data were obtained only on two donors and the high natural variability in background chromosome breakage between donors is known, thus limiting the interpretation of results (Repavich et al., 1990)

Acute toxicity

Anatoxin–a and Homoanatoxin-a

Anatoxin–a is a potent pre- and post-synaptic depolarizing agent, acting by efficiently competing with acetylcholine for nicotinic receptors in neuromuscular junctions and in the central nervous system (Aronstam and Witkop, 1981; Campos et al, 2006). The values of LD_{50} are shown in **Table 3.3.3.1**.

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Table 3.3.3.1 Data on ATX acute toxicity expressed as LD_{50} values.

Toxin	LD_{50}	Value	SD (±) 95%CI (range)	Unit	Specie and Notes	References
(+)Anatoxin-a	LD_{50} oral	=16.2	15.4-17.0	mg/kg bw	Mouse	Stevens and Krieger, 1991
(+)Anatoxin-a	LD_{50} i.p.	= 260	240-280	µg/kg bw	Mouse	Stevens and Krieger, 1991
(+)Anatoxin-a	LD_{50} i.p.	=317*	300-335*	µg/kg bw	Mouse. *Value corrected considering pure (+)ATX (386 µg/kg bw/d (365-408) for (+)ATX hydrochloride, test item used)	Valentine et al., 1991
(±)Anatoxin-a	LD_{50} i.p.	= 749*	695-809*	µg/kg bw	Mouse. *Value corrected considering pure (+)ATX (913 µg/kg bw/d (846-985) for (+)ATX hydrochloride, test item used)	Valentine et al., 1991
	LD_{50} i.p.	= 5.3		µg/kg bw	Rat	Cook et al., 1989
Anatoxin-a (likely racemic compound)	LD_{50} i.p.	= 40-60		µg/kg bw	Mouse. Mice died within 30-60 min	Mahmood and Charmichael, 1986
	LD_{50} i.p.	= 228		µg/kg bw	Mouse. Poor reporting, toxin extract contained impurities	Cook et al., 1988
(-)Anatoxin-a	LD_{50} i.p.	> 60*		mg/kg bw	Mouse. *Value corrected considering pure (-)ATX (73 mg/kg bw/d for (-)ATX hydrochloride, test item used). No deaths or clinical signs observed up to this dose. Only 1 animal per dose	Valentine et al., 1991
(+)Anatoxin-a	LD_{50} i.v.	= 85		µg/kg bw	Rat	Adeyemo and Sirén, 1992
(±)Anatoxin-a	LD_{50} i.v.	= 400		µg/kg bw	Rat	Adeyemo and Sirén, 1992

Death subsequent to the administration of a lethal ATX dose in mammals generally occurs within 2-6 min and it is due to muscular paralysis and respiratory failure (Fawell and James, 1994): clinical signs include gasps, tremors and convulsions.

Acute toxicity differs between the stereo-isomers of ATX: the (+)ATX enantiomer shows a higher affinity than acetylcholine and nicotine for the agonist reception site of the nicotinic receptors,

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resulting in a more potent contractive action and in the overstimulation of the muscle (Swanson et al., 1986; Fawell et al., 1999). The toxicity of mixtures of the two ATX-stereo-isomers and that of pure (-) ATX is lower than that of the (+) enantiomer (Valentine et al., 1991). ATX is also able to interact with the muscarinic receptors but binding seems to be less selective (Osswald et al, 2007). Direct intravenous (i.v.) injection results in the highest toxicity (Fawell et al., 1999). The i.p. LD_{50} of the pure (+) enatiomer in mice is 260-317 µg/kg bw (Stevens and Krieger, 1991; Valentine et al., 1991) whereas after oral administration the LD_{50} is higher than 16000 µg/kg bw (Steven and Krieger, 1991).

The acute toxicity of homo-ATX is similar to that of ATX (i.p. LD_{50} = 250 µg/kg bw in mice) with the same symptoms and death within 7-12 min (the data was obtained on purified toxin but the record was excluded from data extraction due to very poor reporting) (Skulberg et al., 1992). When given orally by gavage, homo-ATX in extract was 10 times less toxic (record excluded from data extraction because obtained with cyanobacterial extracts) (Lilleheil et al., 1997).

Anatoxin-(a)S

Anatoxin-a(s) (ATX-s) irreversibly inhibits acetylcholinesterase (AChE) in the neuro-muscular junctions, a mechanism similar to that of carbamate and organophosphorous insecticides, to which ATX-s is structurally related (Carmichael and Falconer, 1993). By blocking hydrolysis of the neurotransmitter, ATX-s determines acetylcholine accumulation, leading to nerve hyper-excitability. Anticholinesterase activity of ATX-s is limited to the peripheral nervous system: indeed, the brain and retinal AChE activities remain normal even in lethally poisoned animals. The affinity for the human AChE in red blood cells is relatively high; therefore the risk of an acute intoxication for humans is higher than for some aquatic species (Carmichael et al., 1990). The acute neurological effects in mammals are muscle weakness, respiratory distress (dyspnea) and convulsions preceding death, which occurs due to respiratory arrest. Viscous mucoid hypersalivation is a typical symptom induced of ATX-s: the "s" in the name stands indeed for "salivation".

The LD_{50} in mice after i.p. injection is in the range from 20-40-60 µg/kg bw (surviving time 5-30 min) (Mahmood and Carmichael, 1986; Matsunaga et al., 1989) to 228 µg/kg bw (Cook et al., 1988). In rats a lower i.p. LD_{50} of 5.3 µg/kg bw is reported (Cook et al., 1989). Data on oral administration are not available.

Additional effects attributed to ATX on the basis of some *in vitro* studies (which were not included in the data extraction data base for the time being) include cytotoxic effects, caspase activation and apoptosis in mammalian cell cultures (Rao et al, 2002). Induction of oxidative stress with alteration of a panel of related enzymatic activities (i.e. superoxide dismutase, catalase, glutathione-S-transferases and reductase), formation of reactive oxygen species and increased lipid peroxidation have been evidenced.

Subacute/subchronic toxicity

Anatoxin–a and Homoanatoxin-a

For repeated ATX toxicity, the available studies are reported in **Table 3.3.3.2**.

The key studies are the oral ones: 28 day-treatment of mice by gavage (0, 98, 490 and 2460 µg pure ATX/kg bw per day; 10 animal per group; Fawell et al., 1999) and 54 day-treatment of rats *via* drinking water (0, 51 and 510 µg ATX/kg bw per day; 20 females per group; Astrachan et al., 1980).The latter only reported that no effects were seen in treated animals but no quantitative data were reported (likely due to the negative results obtained). There is also an indication coming from maternal toxicity in a teratogenicity study, but it is known that parameters checked in these kind of

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Table. 3.3.3.2 Data available on ATX repeated toxicity

Toxin	Reference value	Duration	Value	Unit	Specie and Notes	References
Oral						
(+)Anatoxin-a	NOAEL	28 days (gavage)	= 98*	µg/kg bw/day	Mouse. (*) Value corrected by authors considering pure (+)ATX (120 µg/kg bw/d for (+)ATX hydrochloride, test item used). 490 and 2460 µg/kg bw/d were the other two tested doses.	Fawell et al., 1999
(+)Anatoxin-a	NOAEL	9 days	= 2460	µg/kg bw/day	Mouse. Maternal toxicity in a study aimed to teratogenicty. Value corrected by authors considering pure (+)ATX (3 mg/kg bw/d for (+)ATX hydrochloride, test item used)	Fawell et al., 1999
Anatoxin-a	NOAEL	54 days (Drinking Water)	= 510	µg/kg bw/day	Rat. Unbound NOAEL: no effect seen at the highest dose tested.	Astrachan et al., 1980
Intraperitoneal						
(±)Anatoxin-a	NOAEL	7 days	=50	µg/kg bw/day	Mouse. The paper focuses only on testes toxicity . The NOAEL is derived from histopathological findings observed only at the high and mid dose.	Yavasoglu et al., 2008
(±)Anatoxin-a	LOAEL	7 days	= 50	µg/kg bw/day	Mouse. The LOAEL is derived due to reduced sperm counts in all treatment group. However hystopathological findings are observed only at the high and mid dose.	Yavasoglu et al., 2008
Anatoxin-a	NOAEL	5 days	125	µg/kg bw/day	Mouse. Maternal toxicity	Rogers et al., 2005
Anatoxin-a	NOAEL	21 days	16	µg/day	Rat. Unbound NOAEL: no effect seen at the dose tested. Qualitative description of data	Astrachan et al., 1980

studies are limited. No clear effect (clinical signs, body and organ weight, haematology and clinical chemistry, whole blood and brain cholinesterase activity, histological examination) was observed even at the highest doses tested, therefore a clear NOAEL could not be derived. However, in the study on mice, two deaths were reported in the high and mid-dose group (Fawell et al., 1999). The authors

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could not determine the cause for either deaths and they not completely ruled out that the observed deaths could have been treatment–related. Therefore, adopting a very conservative approach, the NOAEL was taken to be the lowest of the dose levels, that is 98 µg pure ATX/kg bw per day. However uncertainties related to this interpretation are quite high.

The striking difference between the i.p. and the oral acute toxicity strongly limits the meaning of data obtained by the parenteral route of exposure, for extrapolation to humans.

No data are available on HTX.

Anatoxin-(a)S

No data are available.

Chronic toxicity and Carcinogenity

No data are available for the three toxins.

Reproductive/developmental toxicity

Anatoxin–a and Homoanatoxin-a

In male mice i.p. treated with racemic (+/-) ATX (50, 100 and 150 µg/kg bw/d) for 7 days, the toxin caused dose-related effects, as degeneration of the seminiferous tubules, vacuolisation in Sertoli cells, loss of germ cells and consequently reduced sperm counts (all doses); histopathological findings were observed only at the high and mid dose (Yavasoglu et al., 2008).

In teratogenicity/developmental study mice were treated i.p. with 125, 200 µg/kg bw ATX (GD 8-12 or 13-17): no foetal malformation and reduced viability were observed even in the presence of maternal toxicity (at 200 µg/kg bw decrease of motor activity) (Rogers et al., 2005).

Some effects in hamster foetuses were described when dams were i.p. treated 3 times per day GD12-14 with 125, 200 µg/kg bw ATX: one litter of 10 embryos from the 125 µg/kg bw group showed hydrocephaly and presence of stunted foetus with normal morphologies except for size, in the absence of maternal toxicity (no effect at the higher dose) (Astrachan et al., 1980).

No data are available for HTX.

Anatoxin-(a)S

No data are available.

Epidemiological data in humans

No data are available for the three toxins.

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3.3.4. Cylindrospermopsin

The cylindrospermopsin (CYN) molecule consists of a tricyclic guanidine group combined with a hydroximethyl uracil (Figure 3.3.4.1). Several variants exist such as the 7-epi-CYN and the 7-deoxy-CYN.

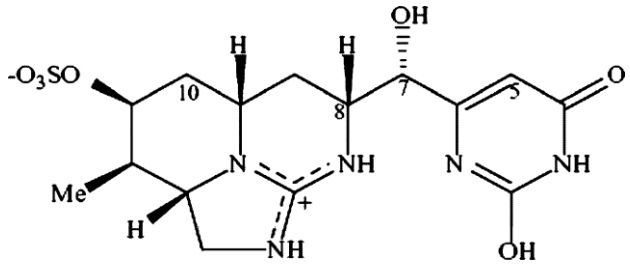

Figure 3.3.4.1 Chemical structure of cylindrospermopsin.

ADME

Cylindrospermopsin is highly hydrophilic; its intestinal absorption needs active transport systems as well as the entrance into hepatocytes, making use of the bile acid transport system (Chong et al., 2002). However, due to the small size of the molecule, a limited passive diffusion through biological membranes occurs, as indicated by in vitro studies, showing cytotoxic effects in a cell line, not expressing bile acid transport system (Chong et al., 2002). When [^{14}C]-CYN (0.2 mg/kg bw) was i.p. injected to mice, radioactivity was recovered mainly in the liver and, to a lesser extent, in the kidney (20.6% and 4.3% of the administered dose 6 hours after the treatment, respectively).

Urinary excretion was similar at 0-12h and 0-24h (25%) and negligible after 24 h. The faeces contained 26.9% at 0-12 h, then the faecal excretion was negligible. Considering also the content of tissues at 48h the recovery was 85-90%. At 48h in kidneys or spleen lesser than 1% was found. The mean recovery from livers was 7.7% (range = 4.7-23.2%) (Norris et al., 2001).

Most of the urinary radiolabel (about 72%, corresponding to around 50% of the administered dose) was associated with the parent compound (Norris et al., 2001), indirectly indicating CYN can undergo biotransformation.

Experimental evidences, produced mainly *in vitro* (not considered for data extraction for the time being) suggested that CYN is bioactivated by cytochrome P450 (P450). Support for the involvement of the CYPs in the bioactivation of CYN is provided by Shaw et al (2000) who demonstrated that a main target of cylindrospermopsin toxicity is the periacinar region of the liver, an area where substantial CYP450-mediated xenobiotic metabolism occurs. The substantial GSH depletion, observed after CYN oral administration to rats, leads to the hypothesis that CYN can be further metabolized by GSH conjugation. Nevertheless, the reduction in GSH content may be also attributed to the inhibition of GSH synthesis. However, it was published during the preparation of this report (and therefore outside the time-frame of the ELS associated to this procurement) that no metabolic transformation can be detected *in vitro* with CYN using rodent and human liver fractions as well as a metabolically competent human hepatic cell line (HepaRG) (Kittler et al., 2015). However, in the same paper it was reported that treating cells with ketoconazole, a known CYP3A4 inhibitor, the cytotoxicity is significantly decreased: the mechanism of this effect should be further investigated.

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Genotoxicity

In vitro, with bacteria, no genotoxicity response in the Ames test was observed. Neither an increase in the number of revertants nor an inhibition of the growth of bacteria with or without metabolic activation (Sieroslawska, 2013).

In vitro on mammalian cells, several studies are available evidencing a dose dependent increase in DNA damage. Using different chemical inhibitors of CYP450, the prevention of DNA damage was evident (Humpage et al., 2005). No chromosome aberrations were detected in CHO-K1 cells after the exposure to CYN with or without metabolic activation (Lankoff et al., 2007; Fessard and Bernard, 2003). In contrast CYN induced MNi formation in two human cell lines HepaRG (hepatic origin) and Caco-2 (from colon carcinoma) both metabolically active (although Caco2 at very low levels) (Bazin et al., 2010a).
With Caco-2 cells cytotoxicity was dose dependent and higher in differentiated cells vs non differentiated ones; MNBNC increased to a maximum at 1.5 µg CYN/ml in both differentiated and undifferentiated cells. Ketoconazole treatment of undifferentiated cells reduced genotoxicity to 50% but cytotoxicity showed much lower decrease. With HepaRG MNBNC increased to a maximum at 0.06 µg CYN/ml in differentiated cells, while with the undifferentiated no significant difference with control was observed. In the presence of ketoconazole no micronucleus induction and significant protection from CYN cytotoxicity at all concentration was observed (Bazin et al., 2010a).
CYN induces DNA strand breakage also *in vivo* in mice. In i.p. injected mice, increased DNA damage was observed only in the colon (100 and 200 µg/kg bw). After oral exposure DNA damage was observed in the bone marrow (1 and 2 mg/kg bw) and colon (4mg/kg bw) (Bazin et al., 2010b), although the treatment doses were quite high (inducing high levels of cytotoxicity, which is always associated to DNA damage), thus limiting the validity of the study. The genotoxicity of CYN (or of its metabolites) is therefore still controversial.

Acute toxicity

It is considered a cytotoxin, since it produces hepatotoxic and nephrotoxic effects but other organs are also affected (Falconer et al., 1999; Seawright et al., 1999; Terao et al., 1994). Data of sufficient quality are available on crude extracts or freeze-dried cells, as shown in **Table 3.3.4.1.**

Although data did not meet the inclusion/exclusion criteria, since they were the only available, it was decided to include them in the inventory, using 'notes' to highlight the limits they are affected by. It is reported by most review papers that the i.p. LD_{50} of pure CYN in mice is 2.1 mg/kg bw after 24 h, 0.2 mg/kg bw when the observation period is prolonged to 120–144 hours, referring to a paper dealing with the purification method for CYN (Ohtani et al., 1992). The information is added as a 'note' of the cited paper, without any further detail, therefore the reliability of the information could not be checked, although in line with results obtained with extracts.

Cylindrospermopsin-containing extracts have a late and progressive acute toxicity: death usually occurs between 24 and 120 hours after treatment, and LD_{50} in mice depend on the duration of the observation time. When mice were dosed with freeze-dried extracts from *C. raciborskii* via the oral route, the acute toxicity was lower when compared to the i.p. treatment (Seawright et al., 1999, Falconer et al., 1999).

Acute hepatic damage is localized in the centrilobular areas, being characterized by hepatocyte vacuolization and increased pigmentation of nuclei and cytoplasm. Necrosis and increased lumen of proximal tubules and alterations in the glomerulus are the main features of renal toxicity (Falconer et al., 1999).

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Table 3.3.4.1 Data on CYN acute toxicity expressed as LD_{50} values.

LD_{50}		Value	Unit	Specie and Notes	References
LD_{50} oral	range	4400-6900 (2-6 days)	μg_{equiv}/kg bw	Mouse. Non purified extract with very low content of toxin (0.2%)	Seawright et al., 1999
LD_{50} i.p.	range	100-352 (24h) 45.5-189 (7 d)	μg_{equiv}/kg bw	Mouse. Non purified extract with content of toxin different in the used batches. LD_{50} recalculated considering the toxin content. (not recalculated $LD_{50:}$ range 50- 110 (24h), 20-65 (7d) mg dry weight of lysed cells per kg.	Falconer et al., 1999
LD_{50} i.p.	=	116	µg crude extract/kg bw	Mouse. Crude extract without toxin's quantification	Banker et al., 1997

The mechanism of action of CYN as such has been associated with inhibition of protein synthesis (Terao et al., 1994), whereas metabolites very likely act with a different mechanism (Froscio et al., 2001). Indeed, in mice hepatocytes, CYN concentrations ≤0.5µM were able to inhibit protein synthesis in 4 hours; necrosis occurred only at 10-fold higher concentrations (in 18 hours). The presence of P450 inhibitors prevented cell death (observed in 18 hours) but not protein synthesis inhibition. This suggest that at low CYN concentrations toxicity is mainly due to the parent compound through protein synthesis inhibition, whereas at higher concentrations the toxicologically relevant compounds are very likely represented by CYN metabolites (Froscio et al., 2003).

Subacute/subchronic toxicity

Among the available repeated toxicity studies, only one has been conducted with the pure toxin orally administered to mice: animals were treated for 11 weeks by gavage with purified CYN (0, 30, 60, 120, and 240µg/kg/day bw) (Humpage and Falconer, 2003). The treatment resulted in a dose-dependent increase in liver and kidney weight, alteration in plasma enzymes (used as markers for hepatic and renal toxicity), and consistent histopathological changes at the high doses. The renal effects occurred at lower doses: a NOAEL of 30 µg/kg bw per day can be identified on the basis of increased kidney weight observed at the immediately higher dose tested (i.e., 60 µg/kg bw per day- dose at which also the urinary protein content was altered) (Humpage and Falconer, 2003).

Other studies are available with mice treated with 3 doses of *C. raciborskii* extracts dissolved in drinking water (estimated to contain 0, 216, 432, and 657µg toxin equivalents/kg/day bw) for 10 weeks: no NOEL could be derived, since effects on kidney and liver were evidenced in all treated animals at each dose. The result suggests that cyanobacterial constituents other than CYN may contribute to toxicity (Humpage and Falconer, 2003).
An additional subchronic study is available, describing oral toxicity in mice exposed to CYN in drinking water for 42 weeks (Sukenik et al., 2006): results qualitatively support the already mentioned findings, confirming the liver and the kidney as the major targets for CYN-induced toxicity. However, the study design suffers some major flaws and was not considered suitable for data extraction.

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Table 3.3.4.2 Data on CYN repeated toxicity.

Duration	Reference value	Value	Unit	Specie and Notes	References
Oral					
11 weeks	NOAEL	= 30	µg/kg bw/d	Mouse. Considering the organ weight as the most reliable indicator of adverse changes See the text	Humpage and Falconer, 2003
Intraperitoneal					
5 days	NOAEL	= 16	µg/kg bw/d	Mouse. Maternal toxicity.	Rogers et al., 2007

Chronic toxicity and Carcinogenicity

No in vivo reliable data with pure CYN are available. Indeed some preliminary data (Falconer & Humpage 2001), questionable due to flawed study design, were never confirmed.

CYN transforming activity was assessed in the Syrian hamster embryo (SHE) cell. Cell transforming potential was found at very low, not cytotoxic, CYN doses (10-7 to 10-2 ng/ml), while at higher concentrations cell transformation was not observed (Maire et al., 2010).

Reproductive/developmental toxicity

After oral treatment at doses in the range of 0.03-3 µg/kg bw for 20 days, no significant differences in body weight gain was observed. There was absence of alterations in all parameters of reproductive performance study and absence of malformations in the foetus (de Almeida et al., 2013).

Repeated i.p. administration of CYN doses in the range 8-128 µg/kg bw to mice during GD 8-12 evidenced maternal toxicity at 50 µg/kg bw (vaginal bleeding and blood in tail tips hepatocytes necrosis and kidney lesions, alterations of haematological parameters) and lethality (62%); indeed lethality of a significant number of dams was reported at doses ≥ 32 µg/kg bw (16 µg/kg bw was the first dose without maternal death: this value was considered as the NOAEL for maternal toxicity). A reduction in litter size occurred in treated groups but pups at PND 6 showed normal development (Rogers et al., 2007). No adverse effects on foetal weight or incidence of anomalies were observed in foetus from surviving dams (Rogers et al., 2007).

The same dose used to treat mice i.p. at GD 13-17 resulted in a reduction in litter size in treated groups. Dams exhibited low lethality (3%) but similar toxicity at the GD 8-12 group but the haematological parameters returned to normal 7 days after the last dose (Chernoff et al., 2011, 2014). Dams gave birth significant earlier than controls. A significant number of pups were found dead and incidences of blood in the gastrointestinal tract and hematomas in the tips of the tails were reported in survivors. On PND 5 the litters had significantly reduced weight gain and viability.

In a follow up study, exposed male pups (n=5) still weighed less than controls after 15 months reaching the statistical significance only at the last time point (PND 344 and 461) (Rogers et al., 2007).

Epidemiological data in humans

In the poisoning episode of dialysed patients in Brazil, the presence of CYN in contaminated water was also reported, possibly contributing to some of the observed effects. However, data usually refer to MCs and it is not possible to quantify the CYN contribution (Carmichael et al., 2001).

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3.3.5. Saxitoxins

Saxitoxins (STX) are a group of toxins known as potent neurotoxins produced by some marine dinoflagellate genera and also by freshwater cyanobacteria and can cause in humans a syndrome known as paralytic shellfish poisoning (PSP), characterized by symptoms varying from a slight tingling or numbness to complete respiratory paralysis.

These natural alkaloids are a family of more than 30 molecules, consisting of a tetrahydropurine group and 2 guanidine subunits. The family can be subdivided into four groups, depending on the substitutions in the variable positions R1 to R4 (see Figure 3.3.5.1):

1. saxitoxins and neosaxitoxin, nonsulphated molecules whose structure is similar to carbamates (STX, dcSTX, neoSTX),
2. mono sulphated gonyautoxins (GTX 1 to 6 and dcGTX 2 and 3),
3. doubly sulphated C-toxins (C1-2), and
4. variants identified in strains of *Lyngbya wollei* (LWTX 1-6) from the USA.

Alternatively they are grouped on the basis of the R4 substituent into carbamate toxins (STX, neoSTX and GTX1-4), sulfamate toxins (GTX 5-6, C1-4) and decarbamoyl toxins (dcSTX dcneoSTX, dcGTX1-4).

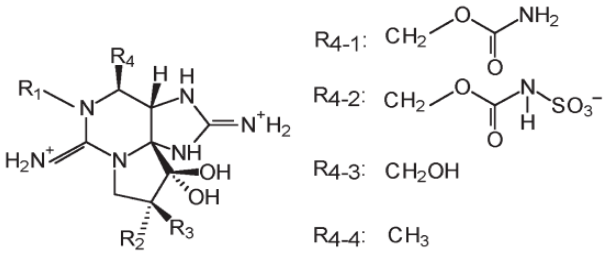

Figure 3.3.5.1. General structure of STX congeners. R4-1: carbamate toxins, including STX and neo-saxitoxin; R4-2: N-sulfocarbamoyl (or sulfamate) toxins, including GTX5 and GTX6; R4-3 decarbamoyl toxins, including dcSTX.

Toxicological data reviewed in the EFSA opinion from the scientific panel on contaminants in the food chain (CONTAM) (EFSA, 2009a)

The Scientific Opinion of the EFSA CONTAM Panel from a request from the European Commission on Marine Biotoxins in Shellfish – Saxitoxin Group (The EFSA Journal (2009) 1019, 1-76) had evidenced that the toxicological database for STX-group toxins was limited and mostly studies concerning their acute toxicity following intraperitoneal administration.
In order to estimate the total toxicity of the seafood, the toxicity equivalency factors (TEFs) approach, which permit to express the toxicity of a mixture of PSPs in terms of STX equivalents, allowed the EFSA Scientific Panel to propose the following TEFs, based on acute i.p. toxicity in mice: STX = 1, NeoSTX = 1, GTX1 = 1, GTX2 = 0.4, GTX3 = 0.6, GTX4 = 0.7, GTX5 = 0.1, GTX6 = 0.1, C2 = 0.1, C4 = 0.1, dc-STX = 1, dc-NeoSTX = 0.4, dc GTX2 = 0.2, GTX3 = 0.4, and 11-hydroxy-STX = 0.3.

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However, it was stated that further toxicological data were needed for the establishment of robust TEFs for the most frequently occurring analogues of STX by the oral route of administration.

Based on available information, EFSA Scientific Panel concluded that the major molecular mechanism of action of STXs and derivatives on nerves and muscles fibres occurs by their interaction with voltage-gated sodium channels, resulting in blockade of ion conduction and generation of action potentials, leading to progressive loss of neuromuscular function and ultimately muscular paralysis.
Since no data on the chronic effects of STX-group toxins in animals or humans were available, the EFSA Scientific Panel could not establish a tolerable daily intake (TDI).

From the available case reports, involving several hundred cases of human intoxications, a lowest-observed-adverse-effect-level (LOAEL) for PSP of around 1.5 µg STX equivalents/kg bw could be established. Furthermore, since many individuals did not suffer adverse reactions at much higher intakes, it could be concluded that this LOAEL was very close to the threshold for effects in the most sensitive individuals, and a factor of 3 was applied to the LOAEL, in order to estimate a no-observed-adverse-effect level (NOAEL) of 0.5 µg STX equivalents/kg bw Therefore, in view of the acute toxicity of STX-group toxins, the Scientific Panel established an acute reference dose (ARfD) of 0.5 µg STX equivalents/kg bw

The available information made it difficult to draw definite conclusions on the estimate of exposure, since there were many uncertainties related to the human case reports: samples of the meal associated with illness were analysed in some cases, while in other cases toxin concentration was determined in uncooked shellfish; moreover, samples were collected on the same day of the incident in some cases, while in others they were collected on a different day. Further uncertainties were related to the STX stability during cooking, since the toxins are only partially destroyed by high temperatures of cooking and are, at least in part, poured into the cooking fluids, which may still be ingested, for example when eating chowder. Therefore, the exact incidence of different cooking and eating practices on toxin levels and exposure remained uncertain.
Because STXs have acute toxic effects, the EFSA Panel had established 400 g of shellfish meat as a high portion size to be applied in the acute risk assessment. Consistently, the consumption of a 400 g portion of shellfish contaminated by saxitoxins resulted in an intake of 320 µg toxin (equivalent to 5.3 µg/kg bw in a 60 kg adult), applying the current EU limit of 800 µg STX equivalents/kg shellfish meat.
Since this intake was much higher than the ARfD of 0.5 µg STX equivalents/kg bw (equivalent to 30 µg STX equivalents per portion for a 60 kg adult), this represent a critical concern for the health of the consumers. This means that a 400 g portion of shellfish should not contain more than 30 µg STX equivalents corresponding to 75 µg STX equivalents/kg shellfish meat, in order not to exceed the ARfD of 0.5 µg STX equivalents/kg bw
The EFSA Panel had also pointed out that a reliable and representative estimation of dietary exposure to saxitoxins could not be definitively drawn, because of considerable differences in toxin profiles, different number of analogues determined and diverse limits of quantification/detection of analytical methods applied, and frequent cases of non-quantifiable samples.
The mouse bioassay (MBA) and the Association of Official Analytical Chemists (AOAC) HPLC method were elected to be used in official analysis for the detection of saxitoxins, being both capable to detect saxitoxins at the EU regulatory levels of 800 µg STX equivalents/kg shellfish meat. However, the limit of detection of MBA is of approximately 370 µg STX equivalents/kg shellfish meat, while in the AOAC-HPLC method it is strongly affected by the different toxin profiles, therefore the EFSA Panel warned that every reductions of the regulatory limits for STXs detection would make it necessary to modify and subsequently re-validate the AOAC-HPLC method, in order to reduce its limits of quantification.

Update from the literature

The extensive literature search from 2009 onwards pointed out that some new relevant information have been published in recent studies, although several aspects still remain insufficient for a complete characterisation of the toxicological profile of the STX-group. A large number of papers were screened and considered to be irrelevant with respect to the toxicity of the STXs-group, describing mostly

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analytical methods or occurrence of STXs. Relevant publications meeting the inclusion/exclusion criteria are described below.

ADME

A metabolic pathway was proposed for the route of metabolization and detoxication of PSPs in a study *in vitro* by Garcia et al. (2010) using human liver microsomes, showing for the first time direct evidence for the sequential oxidation and glucuronidation of PST *in vitro*, both being the initial detoxication reactions for the excretion of these toxins in humans. In the study, the apparent Vmax and Km formation values for the respective glucuronides of neoSTX, GTX3/GTX2 epimers and STX were determined. The Vmax formation values for glucuronic-GTX3/GTX2, glucuronic-neoSTX and glucuronic-STX were respectively 6.8 ± 1.9 x 10^{-3}, 8.3± 2.8 x 10^{-3} and 9.7 ± 2.8 x 10^{-3} pmol/min/mg protein. The Km of the glucuronidation reaction for GTX3/GTX2 epimers, neoSTX and STX were respectively 20.2 ± 0.12; 27.06 ± 0.23 and 32.02 ± 0.64 µM.

Genotoxicity

Cytotoxicity, genotoxicity and oxidative stress of a mixture of 97% saxitoxin, 3% neosaxitoxin andgonyautoxin was assessed *in vitro* with primary culture of neurons isolated from tropical freshwater fish species *Hoplias malabaricus* by da Silva et al. (2014), using a concentration of 3.0 mg/L. STXs increased specific activity of glutathione peroxidase and lipoperoxidation levels and gave positive results in the comet assay.

Acute toxicity

The paper by Munday et al. (2013) investigated the use of the Toxicity Equivalence Factors (TEFs), currently employed in risk assessment, which is based on the mouse bioassay (MBA) method and, knowing the specific activity of STX itself, permit the expression of the activities of specific derivatives in terms of STX equivalents. It was already pointed out by EFSA that this approach implies a large number of uncertainties: firstly, in the MBA STXs are administered by i.p. injection, which is a non-relevant route of exposure to saxitoxins for humans, being the oral route the most significant one; secondly, no correlation could be drawn between the specific activities determined in the MBA bioassay and the median lethal doses (LD_{50}) of the test compounds; thirdly, there is no evidence that the shape of the curve describing the relationship between dose and death time for all the STX derivatives is the same as that for STX itself.
Since the inadequacy of the i.p. administration was already pointed out by EFSA, the acute toxicities of STX, neoSTX, dcSTX and mixtures of GTX-1&4 and GTX-2&3 were determined in the study by Munday et al (2013), by using two methods of oral administration (gavage and voluntary-dietary-consumption), and compared these data with the acute toxicities obtained by i.p. injection.
The following TEFs were derived in this study: STX = 1, neoSTX = 1.16, dcSTX = 0.64, GTX1&4 = 1.02, GTX2&3 = 0.60, indicating that TEFs, particularly in the case of NeoSTX (assumed as toxic as STX in previous studied based on acute i.p. toxicity), require revision, and that the relative acute toxicities of the test compounds by i.p. injection (expressed as relative LD_{50} values) do not correlate with their relative specific activities by the MBA.
The no acute effect level (indicated as a NOAEL in the paper) of STX, neoSTX, dcSTX, and mixtures of GTX-1&4 and GTX-2&3 after oral administration by gavage were derived, resulting below ranges: STX = 500-560 nmol/kg, NeoSTX = 242-300 nmol/kg, dcSTX = 771-1070 nmol/kg, GTX1&4 = 736-1140 nmol/kg, GTX2&3 = 1130-1270 nmol/kg. Data from the paper by Munday et al. (2013) are reported in Table 3.3.5.1.

Very recently the TEFs for nine PSP analogues were calculated by using automated patch clamp technology to measure interaction *in vitro* with human sodium channels, indicating a large variation of TEFs depending on the channel subtype selected (Alonso et al., 2015).

Repeated (subchronic and chronic) toxicity

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No data on the short and long term repeated toxic effects of STX-group toxins in animals or humans were available in 2009 for the EFSA scientific opinion; a similar data gap could be highlighted at present, except for a paper from Zepeda et al. (2014) which investigated the acute and chronic toxicity of NeoSTX by different routes of administration, showing that only the highest dose studied (6 µg NeoSTX/kg bw) when administered subcutaneously (not relevant for the dietary exposure) induced a reduction of weight and food consumption accompanied by a cholestasic pattern at the end of the treatment period. The effects were completely normalized with the drug withdrawal.

Table 3.3.5.1. LD_{50}, NOAEL and TEF values for some STX variants

Toxin	LD_{50} by i.p. injection (nmol/kg)	LD_{50} by gavage (nmol/kg)	NOAEL by gavage (nmol/kg)	LD_{50} by feeding (nmol/kg)	TEFs
STX	27.8 (24.6-31.2)	1190 (1019-1300)*	544 (500-560)	3200 (2195-4270)	1.00
neoSTX	8.9 (8.0-10.0)	700 (592-803)	276 (242-300)	1260 (1095-1500)	1.16
dcSTX	35.4 (30.7-42.3)	2600 (2460-4220)	890 (771-1070)	8680 (8000-8900)	0.64
GTX 1&4	14.6 (12.6-19.4)	1610 (1480-1660)	820 (736-1140)	3420 (3200-4000)	1.02
GTX 2&3	36.7 (33.7-37.8)	2230 (1870-2560)	1230 (1130-1270)	5590 (5200-6600)	0.60

(*) In brackets the 95% confidence limits are indicated.

3.3.6. Palytoxins

Palytoxin (PlTX) group toxins are marine biotoxins detected in soft corals of the genus *Palythoa* and also in algae of the genus *Ostreopsis* as well as in some edible marine organisms mainly from tropical or sub-tropical areas. Symptoms of poisoning are not completely defined, but include gastro-intestinal illnesses, myalgia, weakness, muscular cramps, dyspnea and sometimes arrhythmias. Some marine cyanobacteria of the genus *Trichodesmium* have been demonstrated to produce PlTX. Independently on the source of production (*Ostreopsis* spp. or cyanobacteria), PlTX can arrive to consumers via the same food chain.

Toxicological data reviewed in the EFSA CONTAM opinion (EFSA, 2009b)

The Scientific Opinion of the EFSA CONTAM Panel from a request from the European Commission on Marine Biotoxins in Shellfish – Palytoxin Group (EFSA, 2009) evidenced that data reported by European countries on occurrence of PlTX-group in shellfish were limited and concerned only PlTX and ovatoxin-A in mussels and sea urchins from contaminated regions not addressed to human consumption, not representing levels of toxins in shellfish meat that currently reach the market.

The molecular responses induced by PlTX are complex, but it was recognized that the primary action is due to their interference with Na+, K+-ATPase ion-pump.
Despite the potential exposure of the human population and its high toxic potential, the EFSA panel evidenced a large deficiency of the toxicological database of PlTX, comprising only acute toxicity studies, and in the absence of long term studies the Panel decided to derive an acute reference dose (ARfD) equal to 0.2 µg/kg bw, based on the LOAEL for oral toxicity (gavage) in mice of 200 µg/kg bw, applying an extra uncertainty factor of 10 in addition to the default uncertainty factors of 10 for intra- and 10 for interspecies variation, since transmucosal transport of PlTX in the mouth after sublingual administration could not be excluded and because mice seem to be less sensitive than other species.
Acute toxicity events of PlTX-group toxins were also reported from human outbreaks, but there were no reliable quantitative data to draw any conclusion on acute toxicity in humans.

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In line with the previous scientific opinions on saxitoxins, a portion of 400 g shellfish meat was considered as an appropriate estimate of a high portion size to be applied in the acute risk assessment, and was stated that this portion should not contain more than 12 µg of PlTX and ostreocin-D, corresponding to 30µg/kg shellfish meat, in order for a 60 kg adult to not exceed the ARfD of 0.2 µg/kg bw.
The limited occurrence data available suggested that the consumption of a large portion of shellfish meat harvested from contaminated regions (therefore not representative of PlTX and ovatoxin-A levels in shellfish meat that currently reach the market) could result in an intake of about 3 µg/kg bw, that is more than 10-fold higher than the ARfD of 0.2 µg/kg bw, supposing for ovatoxin-A a potency similar to Palytoxin.
The MBA was commonly used to detect PlTX-group toxins in fish and shellfish, but since it is considered undesirable for ethical reasons the EFSA Panel highlighted the need of development of alternative assays. If cell based assays seemed to have the lowest limit of detection, they showed in some cases interference with other toxin, while HPLC and LC-MS/MS were indicated as suitable tools for the determination of PlTX-group toxins, so their optimization and validation and the development of reference standards were requested.

Update from the literature

ADME

No toxicokinetics studies on PlTX-group toxins were identified in the literature in our research, except for oral toxicity data indicating a different absorption of PlTX and ostreocin-D in different portions in the gastro-intestinal (GI) tract, being higher in the oral cavity (sublingual administration) than after gavage (Ito and Yasumoto, 2009), as already evidenced in the EFSA Opinion of 2009.

Genotoxicity

No new data available.

Acute toxicity

The toxicological effects of acute oral administration of the palytoxin analog 42-OH-PlTX, which is one of the few congeners available, were evaluated both *in vivo* (mice) and *in vitro* in the paper by Tubaro et al. (2011a), showing a toxicity profile comparable with that of the parent compound palytoxin, with an LD_{50} of 651 mg/kg in line with the range of 510–767 mg/kg body weight evidenced by Munday (2008) and Sosa et al. (2009).
Also the *in vitro* hemolytic activity of 42-OH-PlTX was similar to that of PlTX, with an EC_{50} = 7.6 and 13.2 $x10^{-12}$ M, respectively, in agreement with the hypothesis of a common interaction with the Na^+/K^+ pump, to which they bind with the same affinity (Ciminiello et al., 2009).
The mechanism of toxicity of 42-OH-PlTX was also explored *in vivo* in the paper by Del Favero et al (2014), strengthening the hypothesis that the skeletal muscle is as one of the main target organs for PlTX like toxins after acute oral exposure, as already evidenced in previous studies (Sosa et al., 2009; Tubaro et al., 2011a), and showing *in vitro* a cytotoxic potency similar to that of PlTX; however, in consideration of the limited Ca^{2+}-dependence of the toxic damage induced by 42-OH-PlTX, the authors suggested a specific mechanism of action for this analogue.

Subchronic toxicity

The effects of repeated oral administration of PlTX in mice, which can be representative of human exposure scenarios i.e. through repeated consumption of contaminated seafood at least for a short period of time, were investigated in a study by Del Favero et al. (2013). The study showed that a 7-day-repeated administration of PlTX of doses of 30 mg/kg/day (below the LD_{50}) caused lethality and toxic effects. A NOAEL was estimated at the dose of 3 mg/kg/day, and a LOAEL at the dose of 30 mg/kg/day, with lungs, heart, liver and gastrointestinal tract as the main PlTX targets. However, the

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NOAEL value can be considered as provisional, due to the limited number of animal used, although it can be a valid basis for further testing.
Moreover, some studies (Sosa et al.,2009; Tubaro et al., 2011a) showed ultrastructural modifications of skeletal and cardiac muscles associated to increased plasma level of creatine phosphokinase and lactate dehydrogenase after acute oral PlTX administration and also toxic signs (arrhythmias, electrocardiographic changes) observed in humans, suggesting the cardiac muscle as main PlTXs target (Tubaro et al., 2011b). Based on these findings, a preliminary *in vitro* test was carried out by Del Favero et al. (2013) on primary cultures of rat cardiomyocytes, after histological analysis of mice treated *in vivo* had highlighted alterations and fibre separation in myocardium. The *in vitro* experiments on cardiomyocytes supported the hypothesis of the cardiac damage after PlTX administration (2nM), showing progressive depolarization until complete and irreversible arrest of action potentials in a short time (6 minutes after PlTX application).

Chronic toxicity and carcinogenicity

No new data available.

Epidemiological data in humans

As already evidenced by the EFSA Panel in 2009, no reliable quantitative human data were identified in the literature in our search, since many of the reports were anecdotal and the clinical signs and symptoms were not always well defined; moreover, a relevant number of papers were devoted to the study of the mechanism of action of PlTX.

3.3.7. Ciguatoxins

Ciguatoxins (CTX) are a group of marine toxins which arise from biotransformation in the fish of precursor gambiertoxins produced by the dinoflagellate *Gambierdiscus toxicus*. They are the causative toxins of the Ciguatera fish poisoning (CFP), which is the most common kind of marine food intoxication worldwide that is estimated to annually affect 10.000 to 50.000 people. CFP is a complex syndrome known to result after consumption of tropical or subtropical fish that have accumulated CTX in their tissues, but in the last years these toxins were identified also in fish caught in Europe. Moreover, it has been reported that blooms of some benthic and pelagic species of marine cyanobacteria such as *Trichodesmium* are suspected to be a potential source of CTX, likely entering the food chain of bivalves such as giant clams, molluscivorous and grazing fish (Laurent et al., 2008; Kerbrat et al., 2010). Therefore independently on the source of production (dinoflagellates or cyanobacteria), CTX can arrive to consumers via the same food chain.

Toxicological data reviewed in the EFSA CONTAM panel opinion (EFSA, 2010)

As evidenced in EFSA CONTAM panel on marine biotoxins in shellfish – Emerging toxins: Ciguatoxin group (2010), poisoning in humans, is manifested by gastrointestinal, neurological and, to a lesser extent, cardiovascular symptoms; acute gastrointestinal signs may appear within 6 hours after consumption of contaminated fish, while in cases of neurological symptoms they can take 2-5 days to develop.
The EFSA Opinion had already evidenced the lack of regulatory limits for CTX-group toxins in the European Union (EU). The toxicological database for CTX-group toxins is scanty, comprising only studies on acute toxicity following intraperitoneal (i.p.) administration.
Based on available information, it was concluded that the major mechanism of action of ciguatoxins is related to its direct binding and consequent opening of voltage-gated sodium channels in cell membranes which induces membrane depolarization on nerves and muscle fibres.
Based on their acute i.p. LD_{50} in mice, the EFSA Panel had adopted the following toxicity equivalency factors (TEFs), until better information would be available: P-CTX-1 = 1, P-CTX-2 = 0.3, P-CTX-3 = 0.3, P-CTX-3C = 0.2, 2,3-dihydroxy PCTX-3C = 0.1, 51-hydroxy P-CTX-3C = 1, P-CTX-4A = 0.1, P-CTX-4B = 0.05, C-CTX-1 = 0.1 and C-CTX-2 = 0.3.

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No data on the chronic effects of CTX in animals or humans were available, so the Panel could not establish a TDI. However, due to the very scarce quantitative data both in *in vivo* studies on experimental models as well as related to human intoxications, the Panel concluded that it was not possible to derive an acute reference dose (ARfD).
In line with the approach of FAO (2004), the Panel established a concentration of 0.01 µg equivalents of P-CTX-1/kg of fish as an adequate amount which is expected not to exert effects in sensitive individuals, applying an uncertainty factor of 10 to the lowest concentration 0.1 µg equivalents of P-CTX-1/kg in fish associated with mild symptoms.
The very limited occurrence data of CTX-group toxins in Europe did not allow any exposure assessment on European population.
The mouse bioassay (MBA) was indicated as the most recurring method used for the detection of CTX-group toxins in fish, but since it is considered undesirable for reasons of animal welfare and due to its poor specificity the Panel pointed out the urgency to develop alternative assays. In vitro techniques as well as immunochemical methods give an acceptable detection capability, but do not give information on different toxin congeners, while LC-MS/MS methods, allowing the detection of individual analogues, would be of high value in the quantification of CTX in fish extracts. However, none of the current methods applied for their determination in fish had been validated.

Update from the literature

The update of the available literature confirmed the lack of information on the toxicological database of CTX-group toxins evidenced in EFSA Opinion of 2009. Many studies towards the toxicity of CTX were carried out using cultured or not quantified wild extracts of the epiphytic *Gambierdiscus* spp. (*Dinophyceae*), primary source of ciguatoxins, therefore giving information only on the toxicity of the extracts, not useful to make any conclusion on the pure toxin itself. A number of papers were also excluded from our research since they were focused on the characterization of the precursor gambiertoxins or maitotoxins, which constitute a separate group of toxins isolated from *Gambierdiscus* spp. and characterized by a different mode of action, in line with the EFSA Scientific Opinion of 2009.

ADME
Scarce data were available on the metabolism of CTX-group toxins in animals in the EFSA Opinion of 2009; their biotransformation was inferred in a study by Bottein Dechraoui et al. (2005), since one hour after i.p. injection of C-CTX-1 they were detected in blood; the same authors also evidenced that after injection the blood concentrations of both C-CTX-1 and P-CTX-1 reached a peak between 30 and 60 minutes and rapidly decreased in the first 3-4 hours (Bottein Dechraoui et al., 2008).
Toxicokinetics of the ciguatoxin P-CTX-1 was investigated in rats after intraperitoneal or oral administration in a more recent paper from Bottein et al. (2011). A rapid systemic absorption followed by a bi-exponential decline was observed after a single dose of P-CTX-1, with maximum blood concentrations at 1.97 and 0.43 h after the oral and i.p. dose, respectively. After absorption, they were slowly eliminated from blood, with terminal half-lives estimated to 4 days. Ciguatoxins activity were detectable in liver, muscle and brain 96 h after i.p. and oral administration. The main excretion route was via faeces, since only smaller amounts of CTX appeared in the urine. The faecal excretion of ciguatoxin is an indication of hepatic elimination; however, a possible contribution of non-biliary intestinal excretion may also be considered, due to the lipophilic nature of the toxin. A possible reabsorption of CTX lipid fraction in the kidney or the urinary bladder or a slow or inefficient process of elimination through the kidneys could explain the low levels of toxins found in urine.
Pharmacokinetic characteristics after intravenous administration of P-CTX-1 were performed in rats in a study by Ledreux and Ramsdell (2013), thus yielding a complete set of toxicokinetic parameters for this toxin, together with the TK parameters determined by Bottein Dechraoui et al. (2011).
The study evidenced that the terminal half-life of P-CTX-1 was shorter after the intravenous exposure (36 h) compared to the oral or intraperitoneal exposures (4 days, see above). However, its activity was still present in the blood four days after exposure, although at a level near the detection limit, with a mean residence time of 48.8 h for the intravenous exposure. Since the blood clearance value for intravenous dosing (0.69 mL/h/g) was found to be close to the values determined after oral and

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intraperitoneal exposures, it could be concluded that ciguatoxin elimination rates are the same independently on the route of exposure.

Genotoxicity
No new data available

Acute and repeated toxicity
No new relevant in vivo study was available on acute or repeated-dose toxicity. A moderate number of retrieved papers were related to the mechanism of action leading to the neurological alterations after CTX single/chronic exposure *in vitro*, exploring the molecular changes induced in the expression of genes that control the synaptic transmission (e.g. the glutamate receptor genes).

Epidemiological data in humans
A moderate number of case reports in humans were screened, in which diagnosis of CFP was based above all on the description clinical signs and symptoms, without a reliable quantification of the toxins in the contaminated food, thus having a secondary priority in our research. Some publications reporting epidemiological data were included for the data extraction; however, these studies can be considered of limited value, since some of them were carried out as ecological studies, while the others were retrospective case-control studies, substantially confirming previous findings, as that CFP frequently occurs in tropical or subtropical coastal communities, or with case-patients showing similar symptom complexes and severity as described in other previous studies.

3.3.8. LPS endotoxins

LPS endotoxins are an integral component of the cell wall of all Gram negative bacteria and cyanobacteria; the molecule consists of three regions: an internal acylated glycolipid (termed lipid A), and a central area of liposaccharides, linking the internal subunit with the external specific carbohydrate polymer (O-specific chain).
Among bacteria this external subunit shows the most diversity and is the basis for serological specificity, but also the lipid A moiety is variable. Cyanobacterial LPS endotoxins slightly differ from those typical of other bacteria in the three components, including the presence of small quantities of phosphates.
They have been associated with a series of pathological effects in humans and animal tissues mainly linked to direct contact, including gastrointestinal diseases, skin or eye irritation, skin rashes, allergy, respiratory disease, inflammation, headache, fever, ocular irritation. The few results available indicate that cyanobacterial LPS are generally considered less toxic when compared to LPS of bacteria, such as *Salmonella* (Keleti and Sykora, 1982; Raziuddin et al., 1983).

ADME

No data available

Genotoxicity

No data available

Acute toxicity

Results from maximization tests clearly indicated that different cyanobacterial extracts induce high degree of skin sensitization, independent from the production of intracellular cyanotoxins (Torokne et al., 2001), suggesting a possible role for other cyanobacterial constituents, among which LPS endotoxins. The same is valid for acute and repeated toxicity results obtained with crude extracts when compared to pure toxins.

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Lethality in mice was the most frequent toxicity endpoint tested in many of the papers retrieved. However, most of them were old and gave very few details on the description of procedure and of the results.
In particular, the tested doses were reported in the original papers in terms of dose per mouse; however these doses have been converted to mg/kg body weight for comparison purposes in the review article by Stewart et al. (2006), applying to mice an assumed weight of 20 g, for those studies in which the weight of their animals was not reported. Following this "normalization", an LD_{50} = 2.5 mg/kg was indicated for LPS isolated from *Anacystis nidulans* strain KM in the paper by Katz et al. (1977). However, LPS doses in this case are not fully comparable to those in the other studies, since adrenalectomised mice were used, and it is known that adrenalectomy sensitises mice to the lethal effects of LPS.
LPS extracted from different cyanobacteria in a paper by Keleti and Sykora (1982) showed different lethality in mice, since *Anabaena flos aquae* UTEX 1444 was found to be non-toxic at dose of 250 mg/kg, while LPS from *Anabaena cylindrica* UTEX 1611 and *Oscillatoria brevis* showed, respectively, LD_{50} values equal to 130 mg/kg and 190 mg/kg. LPS from *Microcystis aeruginosa* strains 006 and NRC-1 were found to be active in mouse lethality, and LD_{50} values approximatively equal to 45 mg/kg and 40 mg/kg and were estimated for strain 006 and NRC-1 in a paper by Raziuddin et al. (1983).
In addition to lethality, various other toxicity endpoints were examined, as the local Shwartzman reaction, which is a skin lesion characterized by haemorrhagic necrosis elicited in rabbits, observed for *Anabaena flos-aquae* UTEX 1444 LPS, but not for *Oscillatoria tenuis* LPS, or the rabbit loop assay, resulted negative for *Anabaena flos-aquae* UTEX 1444 LPS in the paper by Keleti and Sykora (1982). However, these results should be only considered as qualitative, since no quantitative measurements of lesions were performed.
In the study from Mayer et al. (2011) LPS from *Microcystis aeruginosa* was shown to stimulate the release of neuroinflammation mediators, as superoxide radicals, cytokines and eicosanoids, and various macrophage inflammatory proteins in rat brain microglia, even if activities were generally only observed at considerably higher concentrations when compared to *E.coli* LPS.
In a study by Jaja-Chimedza et al. (2012) an increase of total glutathione levels as well as of GSH and GSH/GSSG ratio was observed in zebrafish embryos exposed to cyanobacterial LPS, suggesting that perhaps oxidative stress, typical of heterotrophic bacterial LPS, is not involved in the case of cyanobacterial LPS, but an alternative mechanism is more likely involved.
Because of the very limited data on toxicity, it follows that more studies are needed to evaluate the risk associated with the exposure to cyanobacterial LPS toxins.

Repeated (subchronic and chronic) toxicity

No data available.

Epidemiological data in humans

No data available.

3.3.9. BMAA

The cyanobacterial neurotoxin β-N-methylamino-L-alanine (BMAA) has been implicated in the aetiology of neurodegenerative diseases such as the Amyotrophic lateral sclerosis/Parkinsonism-dementia complex (ALS/PDC) and more recently an association of BMAA to ALS and Alzheimer's disease has been proposed although its potential role remains still controversial.
Therefore, current research is focused mainly in the study of the mechanisms by which BMAA leads to motor neuron dysfunction and death, since they are not clearly understood, and of the pharmacokinetic parameters regulating the BMAA uptake into brain, its permeability across the blood brain barrier (BBB) and the metabolic pathways involved.
The major criticisms to a possible involvement of BMAA in the development of ALS–PDC came from the observation that the amounts of BMAA administered to primates to induce some degenerative

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effects (Spencer et al., 1987) were viewed as being the equivalent of a person consuming their body weight of cycad flour every day, therefore corresponding to an unrealistic consumption in humans.

ADME

The database concerning the toxicological profile of this toxin is limited and does not allow making any conclusion. However, concerning the kinetics of its uptake and metabolism, a reasonable number of papers was retrieved and examined. Oral bioavailability, biodistribution and brain uptake of BMAA was early demonstrated by Duncan et al. (1991) after intravenous and oral administration in rats (1991) and primates (1992). The mechanism of BMAA uptake into brain was demonstrated by Smith et al. (1992) in rats using an *in situ* brain perfusion technique, showing that BMAA influx was found to be saturable with a maximal transfer rate (Vmax) of $1.6 \pm 0.3 \times 10^{-3}$ µmol/s/g and a half-saturation constant (Km) of 2.9 ± 0.7 mM. In addition, it was suggested that BMAA was transported into the brain by the large neutral aminoacid carrier (L-system) of the BBB, because uptake was sodium independent and inhibitable by excess L-leucine, but not by L-lysine, L-glutamate or methylamino-isobutyric acid.
More recent studies demonstrate that BMAA could be misincorporated into proteins in rats by protein synthesis-dependent processes and then released and reincorporated into newly synthesized proteins over time (Xie et al., 2013, Karlsson et al., 2014); moreover, the level of protein-associated BMAA in the liver of neonates was found to be more than 10 times higher than that in the brain regions, which may be due to the higher rate of protein synthesis in the liver.
In addition, the secretion of ^{14}C-labeled BMAA into milk in lactating mice and the subsequent transfer of BMAA into the developing brain has been recently demonstrated (Andresson et al., 2013), highlighting that secretion into milk is an important elimination pathway in lactating mothers and an efficient exposure route of BMAA for suckling mice. Moreover, the observation *in vitro* that the influx of BMAA was saturable and had a low sodium dependency in cultured mouse mammary epithelial cells suggested that the amino acid transporters LAT1 and LAT2 are involved in its transport.

Genotoxicity

No data available

Acute and repeated toxicity

BMAA is considered to have a low neurotoxic potency in adult rodents but exposure during the development stages has been reported to cause long-term cognitive impairments and histopathological changes in the adult brain (Karlsson et al.,2009, 2012). Moreover, in a study by Engskog et al. (2013) metabolic profiling of serum from BMAA-treated neonatal rats revealed significant alterations of the serum intermediates metabolites d-glucose, lactate, 3-hydroxybutyrate, acetate and creatine, which are associated with changes in energy metabolism and amino acid metabolism, demonstrating that BMAA induces systemic changes in energy metabolism and amino acid metabolism in neonates (Engskog et al., 2013).

Epidemiological data in humans

Epidemiological studies, mostly of ecological nature (Caller et al., 2009; Masseret et al., 2013), or cases report studies were also retrieved, investigating possible spatial correlations between a chronic exposure to cyanobacterial neurotoxins including BMAA and development of ALS. These studies arose from the observation that there were clusters of ALS at a higher frequency than expected in areas surrounding lakes, lagoon or other sites periodically affected by cyanobacterial blooms (France; New Hampshire, USA). Although these studies had considerable limitations (e.g. they were generally not able to detect BMAA in the collected samples), they supported the hypothesis of a role for BMAA in ALS onset, most likely through ingestion of lake water, consuming fish or inhalation of aerosolized toxins, even if it was not possible to ascertain a direct link between exposure to BMAA and the existence of these ALS cluster.

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3.3.10. Toxins of Lyngbya majuscula

Lyngbya majuscula is a benthic cyanobacterium occurring predominantly in tropical and subtropical coastal waters, and to a lesser extent in temperate regions, which has been found to contain a variety of chemicals that exert a range of biological effects on animals and humans.
In particular, the secondary metabolites of *L. majuscula* lyngbyatoxin A (LA) and debromoaplysiatoxin (DAT) appear to give the most widely common health effects in humans, since they are known to be potent tumour promoters and have a cytotoxic action, producing acute dermatologic reactions, respiratory problems, eye infections, and intestinal haemorrhaging.

ADME

No data available.

Genotoxicity

No data available.

Acute toxicity

Lyngbya majuscula was early identified as the aetiological agent of acute toxic dermatitis since the late 1950s after case reports of acute episodes in Hawaii, which were further supported by more recent models of toxicity. Fujiki et al. (1983) found that LA caused a reddening in the ears in 50% of the mice tested via topical application at the concentration of 0.011 nmole per ear, and that DAT and AT were active at similar levels (0.005 nmole per ear). These results were later confirmed in a study by Osborne et al. (2008), in which both LA and DAT were found to produce in mice ear swelling to a similar extent, and the time course of inflammation and histopathological results were also similar for the two purified toxins. Less than 1 mg per ear of either toxin or a mixture (1:1) of the two toxins gave a measurable increase in ear thickness, and when toxins were combined (1:1) an additive, not synergistic effect was observed.
The intraperitoneal and oral toxicity of *L. majuscula* and purified toxin derived from this organism have been examined in mice (Ito and Nagai, 2000; Ito et al., 2002). LA showed an i.p. lethal dose of 250 mg/kg in immature mice while no deaths occurred in mature mice at 300 mg/kg. With sublethal doses were observed erosion in the stomach, small intestine, cecum and large intestine, as well as inflammation in the lung. No lethality occurred by oral administration of 1000 mg/kg, while at 600 mg/kg p.o. severe mucous secretion and erosion occurred within 60 min in the intestine and within 24 hours in the stomach, and increased inflammatory cells followed these injuries. Effective dose levels and the cause of death were found to be similar to those of aplysiatoxin (AT) poisonings (Ito et al., 2002).

Subchronic and chronic toxicity

No data available.

Carcinogenicty

Both LA and DAT have been shown to have tumor promoting activities (Fujiki et al., 1981, 1983). The proposed mechanism of action of the toxins LA and DAT is via protein kinase C-mediated receptors (Mullin et al., 1990). Consistently, a large part of the literature retrieved contained papers focused on the mechanistic aspects by which tumor promoter substances including LA and DAT exert their action in vivo or in vitro after exposure to a carcinogen initiator agent, not to explore the toxicological profile itself of LA and DAT, being they used in these papers as probe substances.
However, neither skin tumours, nor gastrointestinal cancers have been reported in humans exposed to LA and DAT from *L. majuscula*, and the impact of this tumor promoting activity upon human health is yet unclear.

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Epidemiological data in humans

No data available.

3.4. Mixture toxicity

Individual data coming from the ELS are available in the inventory in Appendix C in tabular format, as MS Excel spreadsheets, which can be can be downloaded from the EFSA website in a Microsoft Excel format together with this report Data were divided in subsets, one for each cyanotoxin.

Cyanobacterial blooms are generally characterized by the presence of multiple toxins, or as in the case of PSP toxins they can be interconverted to other variants once absorbed. Therefore, human exposure to a mixture of cyanotoxins via different routes and media (e.g. drinking water, contaminated food, bathing activity) is very likely. However, the significance of exposure to mixtures of cyanotoxins, potentially via multiple routes and media has not been sufficiently investigated so far.

Although interactions can be expected with other contaminants, based on some information on the MoA, very few relevant papers were selected, regarding possible mixture toxicity between cyanotoxins and other chemicals.

Interactions between cyanotoxins and other cell components/bioactive compounds can occur: indeed, crude cyanobacterial extracts containing known amounts of MC-LR can be more toxic than the equimolar administration of the pure toxin. In addition, concomitant exposure with other chemicals has to be considered. Some examples have started to appear in the literature, although not necessarily referring to human health or carried out with uncharacterised extracts-therefore not meeting the inclusion criteria, such as: (i) different interaction patterns have been reported associated with the combined exposure to some pesticides and MC on *Daphnia* spp. (Asselman et al., 2014); (ii) MC-LR exacerbates genotoxicity of the drinking water disinfection by-product 3-chloro-4-dichloro-methyl-5-hydroxy-2(5H)-furanone in low-dose combined exposure, by enhancing oxidative stress (Wang et al., 2013); (iii) potentiation of gap junctional intercellular communication inhibition associated with the tumour promotion process was observed with mixtures of two anthropogenic contaminants (PCB 153 and fluoranthene) and cyanobacterial extracts (Nováková et al., 2012).

However, since in many cases health based reference values and the toxicological profile of the single toxins are not sufficiently elucidated, the evaluation of possible mixture toxicity is premature and can be, if any, only qualitative. The inventory has been provided as foreseen, but only few studies met the inclusion criteria, not robust enough to draw any conclusions.

3.5. Health-Based guidance values for cyanotoxins

Regarding MC, the only variant for which there are sufficient data to derive a health based reference value is MC-LR, the most studied cyanotoxin. In this case, the WHO used the subchronic NOAEL of 40 µg/kg bw/day, obtained with mice by oral dosing, as Point of Departure (PoD), since human data were not sufficiently robust to be used for the derivation of a health-based value. This approach is supported by the authors of this Report. Indeed, although data of possible effects on male reproductive systems have been more recently reported at lower doses than the above mentioned subchronic NOAEL, they were considered not robust enough for the derivation of a health-based value due to weaknesses in experimental design and reporting of the studies.
The PoD was divided by an uncertainty factor (UF) of 1000, obtained by using a combined factor of 10x10x10, to take into account: (1) inter-species variability; (2) intraspecies variability; (3) lack of lifetime toxicity data (i.e. the NOAEL has been derived from a 13-week toxicity study, corresponding to about 1/6 of the mouse life-span). This additional factor could also include the limited data base on other end-point including the equivocal results obtained with reproductive toxicity using the i.p. administration (available after the derivation of the provisional TDI value by WHO). A provisional tolerable daily intake (TDI) value of 0.04 µg/kg bw/day was derived. This is extrapolated to indicate

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that an adult with a body weight of 60 kg could be orally exposed to 2.4 µg MC-LR per day throughout life, without experiencing any toxicological effect. On this basis, the WHO has derived a provisional Guideline Value (GV) of 1µg/l for the total concentration of MC-LR in drinking water. For the GV derivation, an Allocation Factor (AF) of 0.8 was adopted, assuming that potential daily exposure via drinking water would account for 80% of the total ingestion of MC, via a daily consumption of 2L of drinking water:

$$GV = \frac{TDI \times body\ weight \times AF}{C} = \frac{0.04\ \mu g/kg \times 0.8 \times 60kg}{2\ L} = 1\ \mu g/L$$

(Daily consumption of drinking water (C) = 2 L)

Since oral toxicity data for MC congeners other than MC-LR are not known, the GV has been considered valid for total MC concentrations expressed as MC-LR equivalents.

For shorter exposure periods (which are plausible considering the seasonality of some blooms), by using the same approach and omitting the UF related to study duration, a reference value for subchronic toxicity could be derived equal to 0.4 µg MC-LR/kg bw/day. For short-term exposure period it can also be considered results from the Heinze study (1999), in which MC-LR (commercial product; purity not stated) was administered to 11-week-old male rats in drinking water at doses of 0, 50 or 150 µg/kg bw for 28 days. A dose-dependent increase in relative liver weights (17 and 26% at the low and high doses, respectively) and absolute liver weight was reported, together with significantly increased mean levels of ALP and lactate dehydrogenase (LDH) in both treatment groups (84 and 100% increase in LDH, 34 and 33% increase in ALP in low and high doses, respectively) with no changes in mean levels of ALT or AST observed. The low daily dose tested (50 µg/kg bw) could therefore be considered as a LOAEL (used as a PoD by Health Canada)[14], which divided for 300 as Uncertainty Factor (×10 for intra-species variation, ×10 for interspecies variation, and ×3 for the use of a LOAEL), give a reference value for subchronic toxicity of 0.16 µg MC-LR/kg bw/day.

From MC-LR toxicological data it is also possible to estimate safe concentrations with regard to the acute risk following oral exposure: an acute NOAEL of 25 µg/kg bw, the highest dose at which no hepatic effects are observed can be derived from i.p. injection studies in mice. Considering that the i.p. route is a worst case scenario when compared to the oral route, the application of a correction factor (CF) of 10 may be applied, although still representing a conservative approach. In addition, the default uncertainty factor (UF) of 10x10 = 100 for inter- plus intra-species variability should be applied. This leads to an acute no-effect dose of 2.5 µg/kg bw, corresponding to 150 µg/kg bw for an adult of 60 kg bw. Due to the steepness of the dose-response curve, special attention should be given when the exposure to MC-LR is close to the acute adverse no-effect dose.

The WHO provisional TDI value for MCs, which has hitherto represented the only pragmatic, quantitative approach to the management of risks associated with exposure to this class of cyanotoxins, has some limitations, mainly due to the scarcity of information available on the toxicological profiles of cyanotoxins. Data are available mainly on one single congener (MC-LR) and they are not exhaustive in either kinetic or dynamic respects. The TDI derivation for MC-LR is therefore affected to a high degree by uncertainties. It can be discussed whether or not an additional safety factor should be included to account for the tumour promoting activity of MC, which on the other hand has a threshold, related to the inhibition of hepatic protein phosphatases. In addition, the validity of extrapolation from the acute toxicity ranking obtained via i.p. injection to other routes of exposure, and most importantly among MC congeners, to chronic toxicity remains to be demonstrated. However, despite the uncertainties, the provisional WHO value is still valid, since the data obtained more recently are not robust enough to indicate the need for changing it substantially.

Similar approach has been taken by USEPA and Health Canada in 2015 updating their Drinking Water Guidelines.

[14] Health Canada update of drinking water guidelines for cyanobacterial toxins, available at: http://healthycanadians.gc.ca/publications/healthy-living-vie-saine/water-cyanobacteria-cyanobacterie-eau/index-eng.php

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The WHO has also examined the possible human health implications associated with exposure to cyanobacterial cells and cyanotoxins via bathing activities and has provided provisional guidelines for avoiding the risk from skin irritation and more severe effects. However, the WHO Guidelines for bathing waters do not take into account more recent investigations and present several limitations, including a focus on MCs only.

No other provisional GVs for cyanotoxins have been defined by the WHO so far, since databases for ANTX-a and CYN on repeated, long-term oral toxicity have not been deemed adequate to derive TDIs.

Some tentative derivation have been proposed for ATX, and used for setting limits or guidelines in some Countries, but the levels of uncertainty is even higher than for MC.

Below the example of ATX is described.

Due to the kinetics differences between the i.p. and the oral route, it is considered not fully suitable the use of values obtained with the parenteral administration as point of departure (PoD). Studies characterized by repeated exposure duration and oral route show no 'significant' effect at the highest doses tested, and were therefore not robust enough to derive a solid TDI value.

In fact in one of the studies available (Fawell et al., 1999), the authors of the paper derived a NOAEL =98 µg ATX/kg bw per day, based on the death of two animals in the mid and high group of treatment (210 µg ATX/kg bw), although, beside the uncertainties regarding the causal relationship with ATX treatment, death is not a suitable end-point to be considered for setting a NOAEL. Using this 'very conservative approach' they proposed a GV=1 µg/L for the total concentration of ATX in drinking water from their 28-day study on mice. This threshold would provide an adequate margin of safety (10^3) to protect human health, starting from the derivation of The GV was derived to protect a 10 kg bw child consuming 1L drinking water per day.

Alternatively using the unbound NOAEL, i.e. 510 µg/kg bw/day (Astrachan et al., 1980; 54 days, exposure *via* drinking water, the most relevant exposure route)and applying an extrapolation factor of 100 (10 to take into account interspecies differences, and additional 10 for inter-individual differences in humans) and a further factor of 10 to account for uncertainties in the data base (including the short duration as well as lack of information), a 'minimum Tolerable Daily Intake' (mTDI) would amount to 0.5 µg/kg bw/day. By allocating 80% of the human exposure to drinking water, assuming a consumption of 2 L per day and 60 kg as default body weight for an adult, a 'minimum Guideline Value' for ATX would amount to 12 µg/L (Duy et al., 2000; Funari and Testai, 2008).

The levels of uncertainty associated with CYN is even higher, especially related to its potential but still controversial genotoxicity, associated to its metabolites (not identified so far) and in principle does not allow to derive any provisional reference value. Waiting for clarification of the potential genotoxicity, in order to evaluate the chronic risk associated with its oral exposure, the most appropriate PoD is the subchronic NOEL = 30µg/kg bw/day (Humpage and Falconer, 2003). Similarly to the approach used for MC, dividing the NOEL for a UF of 1000 (100 for inter- and intraspecies variability, and 10 for the limited toxicological data base), a TDI value of 0.03µg/kg bw/day can be derived. This TDI should be updated, once genotoxic and/or carcinogenic properties of this cyanotoxin are demonstrated.

For those toxins already evaluated by EFSA in 2009, those reference values can be used, although some new data suggest that there are indications that they could be updated for STX and PlTX, although information are not yet exhaustive.

In 2009 the EFSA CONTAM Panel (EFSA, 2009) derived an acute reference dose (ARfD) of 0.5 µg STX equivalents/kg bw, that is 30 µg STXeq for an adult of 60kg bw. From the several reports on intoxication episode in humans, affecting more than 500 individuals, the Panel has derived a lowest-observed-adverse-effect-level (LOAEL) around 1.5 µg STXeq/kg bw. Similarly to the previous FAO evaluation, in view of the number of poisoned individuals the human intra-individual variability was considered covered by the available data. Consequently, the ARfD value has been established considering only an assessment factor of 3 (EFSA 2009), due to the use of a LOAEL instead of a NOAEL as PoD.

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To avoid exceeding the ARfD of 0.5 µg STX equivalents/kg bw for a 60 kg adult, a 400 g portion of shellfish should not contain more than 30 µg STX equivalents corresponding to 7.5 µg STX equivalents/100g shellfish edible part. It has been noted that this portion at the current EU limit of 80 µg STX eq/100 g shellfish edible part, would result in an intake of 320 µg toxin (equivalent to 5.3 µg/kg bw in a 60 kg adult) which is higher than the ARfD and therefore give rise to some health concern. However, the lowering of 'legal' limit of 80 µg STX eq/100 g shellfish edible part could be feasible only when shellfish monitoring will be based on official methods (rather than the prescribed MBA or the so-called 'Lawrence method' by HPLC) sensitive enough to reliably detect it.

Based on more recent experimental animal data as supporting (Munday et al., 2013), the use of the acute NOAEL obtained after gavage administration as PoD leads to the derivation of an ARfD of 1.67 µg STX/kg bw and 0.87 µg NeoSTX/kg bw (applying an assessment factor =100). The values are in the same order of magnitude when compared to EFSA and FAO values based on human data, confirming the consistency of the derived values. The dose-response curve for acute effects both in human and in animals is quite steep, indicating the need for caution at exposures levels close to the limit. To overcome the lack of specific limit values for each congener, for regulatory purposes, total concentration of all STX variants can be expressed as STX concentration equivalents, assuming a common mode of action and using a TEF approach based on the MBA data or the in vitro blocking of Na^+ channel in cultured neuronal cells obtained with certified reference substances. This was considered as conservative approach taking STX as the most acutely toxic within the STX family. However, EFSA stated that the evaluation should be supported with further toxicological studies using oral exposure (EFSA, 2009), which is more representative of human exposure. And indeed, after the adoption of the EFSA Opinion it has been evidenced that the TEF values based on MBA are different from those calculated using the oral LD_{50}, according to which the NeoSTX would have a TEF >1, being more toxic than STX (Munday et al., 2013). When oral acute toxicity was considered, the following TEFs were derived: STX = 1, neoSTX = 1.16, dcSTX = 0.64, GTX1&4 = 1.02, GTX2&3 = 0.60. The no acute effect level after oral administration showed this ranking (in increasing order, therefore from the more toxic to the least): neoSTX 2 fold >STX> dcSTX≈ GTX-1&4 >GTX-2&3.
Very recently the TEF for nine PSP analogues were calculated by using automated patch clamp technology to measure interaction with sodium channels, indicating a large variation of TEFs depending on the channel subtype selected (Alonso et al., 2015). On the basis of these more recent results, the relative potency of the different STX variants as defined by EFSA could be revised.

For PlTX the ArfD is at present derived after sublingual administration, according to the EFSA opinion (2009): apart from the confirmation of the acute toxicity for PlTX and its analogue 42-OH-PlTX after oral administration and some studies on MoA in vitro and in vivo, it is interesting to consider a short term repeated toxicity study in mice, which can be representative of human exposure scenarios i.e. through repeated consumption of contaminated seafood at least for a short period of time. The study showed that a 7-day-repeated administration of PlTX of doses of 30 mg/kg/day (below the LD_{50}) caused lethality and toxic effects. A NOAEL was estimated at the dose of 3 mg/kg/day, and a LOAEL at the dose of 30 mg/kg/day, with lungs, heart, liver and gastrointestinal tract as the main PlTX targets. However, the NOAEL value can be considered as provisional, due to the limited number of animal used, although it can be a valid basis for further testing. Moreover, some studies showed ultrastructural modifications of skeletal and cardiac muscles, suggesting the cardiac muscle as one of the main PlTX target (in line with signs observed in humans).

3.6. Environmental factors affecting Cyanobacteria toxicity

Individual data coming from the ELS are available in the inventory in Appendix C in tabular format, as MS Excel spreadsheets, which can be can be downloaded from the EFSA website in a Microsoft Excel format together with this report Data were divided into two subsets, one for MCs and the other one for toxins other than MC.

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3.6.1. Microcystins

After the first screening 408 papers were selected; from the secondary screening 126 have been considered eligible for data extraction and 122 is the final number of papers analyzed (8 papers have not been found). The main reason for this selection is related to the fact that many studies are not conclusive on the effects of the different analyzed variable, especially the ones carried out in the field. Of the 122, 81 papers reported results from laboratory experiments, 30 reported results of field data analysis and 11 reported a combination of field and laboratory results. It is worth noting that > 80% of paper deals with *Microcystis* sp.

Microcystins are secondary metabolites produced non-ribosomally by the complex gene cluster *mcy*, containing peptide synthetase and polyketide synthase domains (Tillet et al., 2000). The toxicity of a bloom in the field depends first of all on the **genetic composition of the population**, since not all the genotypes possess the gene cluster for the production of toxins. Since the physiological and ecological role of microcystins has not been completely elucidated yet, it is also difficult to understand

Table 3.6.1.1 Classification of papers according to the environmental parameter, the studied variable (MC or mcy abundance or expression) and the kind of experiment.

	Phosphate		Nitrogen		Temperature		Light		other	
	mcy	MC	*mcy*	MC	*mcy*	MC	*mcy*	MC	*mcy*	MC
Field	1	13	4	16	2	3				1
Lab	2	19	4	21			4	18	2	8
Mesocosm	1	1	-	2		1	-	-		

the factors improving the fitness of the *mcy+* genotype vs *mcy-* , favoring the growth of the former *vs* the latter. An important role seems well demonstrated in the carbon-nitrogen metabolism and in redox control, based on the molecular regulation of the *mcy* operon; however neither toxic nor non-toxic strains possess a generally higher fitness, which is rather linked to the particular ecological context (Neilan et al., 2012). The number of studies on the relative success of the toxic genotype are limited (15 papers), also because of the implicit limit in lab experiments in comparing toxic and non-toxic different monoclonal strains which could differ for other unknown phenotypic characteristics, as it is well shown by a paper by Van de Waal *et al.* (2011). In competition experiments, the outcomes from competition experiments between different strains (toxic and non-toxic), were different from the results from experiments where competitors were the *mcyB-* mutant of a toxic wild strain of *Microcystis*.

Temperature seems to positively influence the toxic fraction of the populations both in field and in lab experiments, more than non-toxic fraction (Davis et al., 2009; Dziallas and Grossart, 2011; Li et al., 2014); this result suggests that in a future scenario of global warming, we could expect an increase in the toxic fraction of cyanobacteria population. However, the number of studies is very limited, and refers mostly to *Microcystis aeruginosa*, which is just one of the many toxic species. The only paper looking at toxic fraction in a population of *Planktothrix aghardii* found an increase in the toxic fraction at the decrease of T (Briand et al., 2008). No data are available for phosphorus (**P**) limitation on the toxic cells, and results on nitrogen (**N**) effects are still controversial or depend on the species. In some of the few lab experiments, inorganic N limitation increased the growth of toxic *P. agardhii* (Briand et al., 2008) while decreased the toxic fraction of *Microcystis aeruginosa*, which was stimulated by organic N limitation (Davis et al., 2010). In the field, most of the studies shows a positive correlation between various forms of N and toxic cells abundance, but there are also few examples of negative correlation (N limitation increase the growth of toxic cells). Under condition of **CO_2** limitation, toxic strains are favored when comparing the wild and the non-toxic mutant (Van de Waal et al. 2011; Yu et al., 2015); this suggests a competitive advantage of the toxic cells in an advanced stage of the bloom, when CO_2 starts to be limiting among the cells, especially in the lower layers. In one study on light, under light limitation, the toxic wild type displaced the non-toxic mutant, while in the same condition non-toxic strain won competition with the different toxic strain (Van de Waal et al. 2011).

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The density of the potentially toxic genotype helps explaining a variable percentage of variance in the range of MC concentration in the population. This is because the rate of the toxin gene expression or toxin production can be affected by environmental factors as well, and because toxins can be utilized inside the cell, by forming complexes that are not detected as MC (Zilliges et al., 2011).

MC have a putative role in photosynthetic processes and in metabolism of C and N (Jahnichen et al., 2007; Donald et al., 2011); they can be produced as a stress response, in order to protect some important proteins involved in important metabolic pathways (like RuBiSCO in the photosynthetic one, for example, Zilliges et al., 2011), or can be excreted externally, to function as signaling molecules between cells (Shatz et al., 2007). There is a clear correlation between microcystins production rate and specific growth rate, however many indications from both field and laboratory studies suggest more complex relationships with chemo-physical factors.

Studies from the field cannot give direct information on the mechanisms of production and regulation, and are mostly focused on identifying the most important environmental parameters correlated to MC concentration or production. Their findings are usually based on long time or large spatial series data and/or statistical analysis of large databases (principal component analysis, multiple correlation analysis, etc.). Outcome of field mesocosm studies are related to the trophic state of the water body analyzed. For example, in hyper-eutrophic lakes, more reduced form of N (NH_4 and urea) move the phytoplankton community towards the dominance of toxic strains of *Microcystis*and stimulate the production of MC, therefore increasing the toxicity of blooms (Donald et al., 2011). P enrichment of oligotrophic basin, leads to N depletion and to a decrease in MC concentration (Horst et al., 2014). Few studies on MC variants (containing different atoms of N) suggest that Carbon (C) or N concentration in the media can change their relative contribution (Van de Waal et al., 2009; Van de Waal et al., 2010), but also the exposure to different combination of light and T can change the ratio of MC variants (Rapala and Sivonen, 1998; Rapala et al., 1997; Tonk et al., 2008), thus affecting the toxicity of the bloom.

In many field studies a positive correlation between MC concentration and phosphorus (P) was found. About Nitrogen (N), the results are more controversial, since 8 papers found a positive correlation of MC and N (in various chemical forms) and 4 found a negative correlation. According to several Authors, MC production is constitutive and correlated to growth rate, and MC concentration is correlated to cell density (Long et al., 2001; Oh et al., 2000). Therefore, often cell density correlates with P, N and MC concentration, which seems therefore indirectly influenced by nutrients through their effects on cells growth. Furthermore, P seems to be more related to the energetic state of the cells, and indirectly to MC production, while N seems to have a more direct role in the quantity and the MC production. It is one component of microcystins, and it is more abundant in some variants, therefore the environmental concentration of N can determine the quantity of MC produced and the relative concentration of the different variants. However, since N assimilation and MC production are under common centralized control via NtcA transcription promoter, which can be activated by low cellular quotas of NH^+ to increase active uptake of dissolved or atmospheric N, limitation of N can also activate MC production.

Laboratory experiments provide information on more environmental factors, but also more controversial results. Phosphorus is still positively or no correlated to MC production. Also about N, experiments confirm its complex role in microcystins production. 14 studies found a positive relation between N concentration and MC, vs 6 that found the opposite results and only 1 which found no effects. Looking at *mcy* expression, 1 found a positive relation, vs 3 which found negative relation.

Other parameters that have a role in MC production or mcy expression, are iron (Fe), light and inorganic carbon (all related to photosynthetic carbon fixation). Seven papers tested limitation of iron (Fe) on MC quota/concentration and 2 on *mcy* expression. The concentration of MC increased at Fe limitation in 3 out of 6 cases; *mcyD* expression was stimulated by Fe limitation.

High light intensities (>25 µmol photon/m^2/s) seem to generally decrease the MC concentration (10 papers), if it is the only parameter considered, but in experiments in combination with other factors,

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especially in competition experiments, increasing light favor the production of MC (5 papers). Actually, light seems to stimulate MC production up to a threshold and thereafter to reduce it.

About Carbon and CO_2 limitation, only 2 papers have been considered, but both showed that in condition of C limitation, MC content increased, due to increasing production (Jahnichen et al., 2007) or indirectly, due to the increase of the toxic strain (Van de Waal et al., 2011).

From this brief summary, it is clear that environment plays a very complex role in the toxicity of the bloom, in terms of fitness of the potentially toxic genotype, of regulation of MC production, or MC quota (limitation in the quantity of a necessary nutrient, or influence in the regulation of gene expression). It seems clear that not limiting nutrients are the base for an abundant bloom, which is also the assumption for a risky situation, even if the thresholds can be different in the various species. Less clear is the nutrient role in the toxicity of the bloom. Indeed, limiting N can increase the toxicity of the bloom via more MC production or by shifting the relative ratio of toxic/non-toxic genotype towards higher values.

From field studies, it is still difficult to distinguish direct from indirect effects of the critical parameters.

3.6.2. Toxins other than Microcystins

The number of studies on other toxins are summarized in Table **3.4.2**. With respect to MC, they are much less, and the organisms producing these toxins considered in the studies are more diverse, except for nodularin, produced only by *Nodularia spumigena*, raising more questions than answers.

Table 3.6.2.1 Papers found for toxins other than MCs.

	Nodularin	Cylindrospermopsin	Anatoxin-a	Saxitoxin	BMAA
Field	2	2	2	-	1
Laboratory	10	9	3	5	

For none of these toxins there are studies on factors affecting the genetic composition of the population (toxic/non-toxic cells), even if the genes' complexes have been identified and sequenced for all of them.

For nodularin an important environmental factor is represented by salinity, being *Nodularia spumigena* a brackish species. However, few experiments on salinity are controversial. Nutrient limitation (N and P) seems to increase the production of NOD. An opposite effect has been observed for P in the production of ATX-a, since P enrichment seems to decrease ATX-a concentration. P effect on CYN production is different according to the species: P limitation increase production of CYN by *Aphanizomenon*, while it decreases CYN production by *Cylindrospermopsis sp.*.

Overall, data on these toxins are still really too few to draw any conclusion. Furthermore, also in this case, as for MC, experimental conditions are not standardized and it can be difficult to compare different studies.

3.7. Exposure scenarios

Exposure scenarios to cyanotoxins have been defined comparing cyanotoxins levels reported in the occurrence database with the EFSA Comprehensive European Food Consumption Database (http://www.efsa.europa.eu/en/food-consumption/comprehensive-database). When possible, occurrence data has been classified according to the EFSA 'FoodEx' classification system in the categories of Fish and other Seafood, Food supplements and crops. The category of 'Other edible animals' includes game hunting, birds and livestock.

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As agreed with EFSA, data from laboratory studies as well as those from field studies obtained with non-reliable analytical methods (score 4) have been excluded. In addition only fresh weight data were used, since the conversion from dry weight to fresh weight can be affected by a very high degree of uncertainty, due to different procedure to obtain the dry weight data (often not reported in the available studies).
One of the major problems faced in elaborating exposure scenarios is that of the large number of non-detected samples (e.g. as <LOD or <LOQ) reported in several studies. They represent no 'quantified' concentrations, not necessarily the absence of toxins. In order to use these data, whenever possible, the "bounding" approach has been applied (as adopted by EFSA in its opinions). It consists of attributing to all the samples reported as <LOD or <LOQ the value of zero for the Lower Bound (LB) and the value of LOD or LOQ for the Upper Bound (UB). When no LOD or LOQ were reported in the specific study, the highest LOD/LOQ value available in the occurrence dataset was used.

3.7.1. Microcystins

In the occurrence database the majority of data on MCs (≈83%) relates to the food group "Fish and other seafood" of the EFSA FoodEx classification system, Level 1. The other occurrence data refer to Food supplements (≈26%), game hunting, bird and livestock (≈4%), Crops (≈6%).

In the majority of the studies, MC concentrations are expressed as total, sum of congeners, MC-LR equivalents, more rarely as single congeners. In this report, according to a conservative approach, we have assumed that all the concentrations are expressed as MC-LR equivalents. When data are available only for single congeners, we have calculated their sum. In the diverse studies, data are reported as mean, max or single value. When max values are not available we have roughly estimated them adding the standard deviation to the mean value.

The synthesis of the occurrence data used to define the exposures scenarios to MC in food is presented in Table 3.7.1.1

Table 3.7.1.1 Selected food occurrence data

	n. data (%)	**n. studies**	**N Data <LOD (%)**	**Countries**	**EU Countries**	**HPLC (world wide)**	**HPLC EU**	**ELISA (world wide)**	**ELISA EU**
Fish	*21 (70%)*	8	8(38%)	5	1	5 studies 13 data	0	3 studies 8 data	3 studies 8 data
Sea-food	*8 (13%)*	5	2 (25%)	3	1	2 studies 4 data	0	3 studies 4 data	2 studies 3 data
Other	*2(4%)*	1	0	1	1	0	0	1 study 2 data	1 study 2 data
Crops	*3(6%)*	1	0	1	0	1 study 2 data	0	1 study 1 datum	0
Food suppl	*12 (26%)*	6	2 (17%)	6	3	3 studies 5 data	2 studies4 data	3 studies 7 data	1 study 1 datum

Fish and other seafood

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As shown in table 3.7.1.1, MC occurrence data for the group "Fish and other seafood" have been divided in "Fish" compared with the EFSA FoodEx category (Level 2) "Fish meat" and "Seafood" compared with the EFSA FoodEx categories (Level 2) "water molluscs" and "crustaceans".
The table also shows that only few studies have been conducted in Europe, hence the available data do not allow estimating specific European exposure scenarios.
Some studies report also MC concentrations in fish liver and viscera, with values generally much higher than those in muscle tissues. Yet, they are not normally consumed according to European diet styles and for this reason we have not included them in the exposure scenarios. On the other hand, we cannot exclude that some specific considerations might be done at local level in relation to this potential exposure (e.g., some specific groups of immigrants).
As agreed with EFSA data reported as Dry Weight (DW) have been excluded. Yet, they represent about 58% of occurrence data in fish and other seafood, unfortunately reported without specifying the respective humidity percentages, necessary to derive the corresponding concentrations for fresh weight (FW). Due to the paucity of data on FW, we have also calculated MC concentrations considering DW data, applying a factor of 5, assuming a water content of 80%. We are aware these estimates are rough and not accurate, yet they can help to reinforce the base of data from which deriving useful indications regarding possible risks and data gaps.

Fish muscle

MC Concentration

Table 3.7.1.2 reports MC concentrations in fish muscle expressed as mean, median, maximum and P95 values.

Table 3.7.1.2 MC concentrations in fish muscle from FW data

	All analytical methods		ELISA methods	Chromatographic methods	
	Conc µg/kg LB	Conc µg/kg UB	Conc µg/kg*	Conc µg/kg LB	Conc µg/kg UB
Mean	187	204	81	253	280
Median	10	70	78	0	70
Max value	2860	2860	152	2860	2860
P95	340	340	148	1348	1348

*All the available data are all above the LOD/LOQ.

Consumption in Europe

Considering the EFSA Chronic food consumption statistics for consumers only, the highest 95th percentile of fish meat consumption is that of Spain (265 g/day), followed by Finland (195 g/day), Sweden (159 day), Germany (156.9 g/day), Italy (135 g/day) and France (76 g/day).

MCs intake associated to consumption

Assuming the highest fish consumption (265g/day), MC mean concentrations of 187-204 µg/kg (see table 3.7.1.1), the intake for an adult person of 60 kg bw would be of about 50-54 µg MC/day. These values are well above that of 2.4 µg/day per adult (the chronic safe threshold derived from the TDI of 0.04 µg/day/kg bw, defined in the WHO guidelines (2003, 2004). It can be discussed whether for the general population a chronic daily consumption is a realistic exposure scenario, or this kind of exposure could be limited to local populations (e.g. fishermen and their families). However, the estimated ingestion also exceeds the subchronic reference value (24 µg/day), covering the consumption of contaminated fish for a limited period of time, a scenario which is more realistic also

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considering the seasonality of cyanobacterial blooming. In addition the maximum value (obtained with a chromatographic analytical method) is more than 1 order of magnitude higher than the mean value.

In table 3.7.1.3 data on MC are integrated with concentrations estimated from DW data.

Table 3.7.1.3 MC concentrations in fish muscle considering also DW data

	All analytical methods		ELISA methods	Chromatographic methods	
	Conc µg/kg LB	Conc µg/kg UB	Conc µg/kg*	Conc µg/kg LB	Conc µg/kg UB
Mean	72	80	46	78	89
Median	5	9	14	1	8
Max value	2860	2860	152	2860	2860
P95	178	178	144	181	181

*All the available data are all above the LOD/LOQ.

It can be seen that concentration are approximately ½ with respect to FW data only, but considering the same fish muscle consumption, the intake would of about 20 µg MC/day per adult, well above 2.4 µg/day and similar to the subchronic threshold for no effects (24 µg/day).

In order to draw a more realistic scenario, it is necessary to consider that MC can occur mainly in freshwaters fish species. For the moment information on MC production by cyanobacteria genera in estuarine or marine waters is indeed not available. Out of the 21 data of the occurrence database 10 (48%) refer to the *Cyprinidae* family and the remaining 11 (52%) to 6 different families (1 to 3 data for family).
The 10 MC occurrence data on *Cyprinidae* have been selected form 5 studies in 2 Countries (1 in Europe). Most of them have been determined with ELISA method (80%) and the remaining with chromatographic methods (table 3.7.1.4).

Table 3.7.1.4. Mean, median, maximum and P95 MC concentrations in *Cyprinidae* fish muscle

	Considering only FW data			Considering FW and DW data				
	All analytical methods	ELISA methods	Chromatographic methods (2 data)	All analytical methods		ELISA methods	Chromatographic methods	
	Conc µg/kg	Conc µg/kg*	Conc µg/kg	Conc µg/kg LB	Conc µg/kg UB	Conc µg/kg*	Conc µg/kg LB	Conc µg/kg UB
Mean	351	81	1433	93	96	81	95	98
Median	78	78		8	9	78	4	6
Max value	2860	152	2860	2860	2860	152	2860	2860
P95	1641	148		179	179	148	180	180

*All the available data were all above the LOD/LOQ.

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If also DW data are considered 52 data (68%) refer to the *Cyprinidae* family and the remaining 25 (32%) to 13 different families (1 to 4 data for family).
The 52 MC occurrence data on *Cyprinidae* have been selected form 9 studies in 9 Countries (only 2 in Europe).

The consumption of fish belonging to the *Cyprinidae* family in Europe, according to the EFSA database, is presented in table 3.7.1.5.

Table 3.7.1.5 EFSA data on consumption by European population of *Cyprinidae* Family

Number of dietary survey considered	Countries involved	Numbers of consumers	Mean consumption g/day		P95 of consumption g/day	
			Weighted average	Highest mean value	MIN	MAX
9	8	220	57	167	25	267

As the number of consumers in the dietary surveys is highly variable, to minimize its influence for the "mean consumption" we have calculated a weighted average.

According to the available data on FW, we have calculated a MC intake of about 20 µg/day, based on a concentration of 351 µg MC/kg in *Cyprinidae* fish muscle (mean value considering all analytical method) and the weighted average of mean consumption, 57 g/day. The intake is of about 94 µg/day if the highest 95° percentile of consumption is used .
These MC intakes are above the safe chronic risk threshold and similar to the subchronic one (although it exceeded when considering the 95th percentile).

Lower intakes are calculated when also DW (converted to FW) data are considered. The MC intake is of about 5 µg/day, with a MC concentration of 96 µg/kg (UB mean value considering all analytical method) and the weighted average of mean fish consumption, 57 g/day. The intake is of about 26µg/day if the highest 95° percentile of consumption is used. However, also in this case MC intakes are above the safe chronic risk threshold and close to the subchronic one.

It has to be highlighted that the database is quite poor, and few very high values strongly impacts the overall content and the related elaboration (as shown by the difference between mean, median and P95 values). More data are needed to obtain more robust statistics.

In the worst case, that is a MC concentration of 2860 µg MC /kg and a consumption of 400 g of fish muscle, we would have an intake of 1.14 mg, that is almost an order of magnitude higher than the safe acute risk threshold.

In relation to fish belonging to other families, only 11 data are available for 6 different families (1 to 3 data for family), most of them with values <LOD. The only 2 relevant MC concentrations are referred to *atherinopsidae* (340 µg/kg FW) and *centrarchidae* families (75 µg/kg FW) that lead to intakes of 136 and 30 µg respectively when considering a consumption of 400g of fish muscle.
If also DW data are considered relevant intakes can be reached also consuming fish from *anguillidae* (58 µg) and *gobiidae* (37 µg) families.
It has to be noted that these intakes are based on very few data and the above considerations are not a robust exposure assessment, but have only the meaning to underline the need of further investigations especially on fish families with the highest MC concentrations.

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Seafood

MC concentrations

The data base contains only 8 selected MC seafood concentrations determined either with chromatographic (50%) or with ELISA methods (50%) (see table 3.7.1.6).

Table 3.7.1.6. Mean, median, maximum and P95 MC concentrations in seafood

	All analytical methods		ELISA methods	Chromatographic methods	
	Conc µg/kg LB	Conc µg/kg UB	Conc µg/kg*	Conc µg/kg LB	Conc µg/kg UB
Mean	10241	10258	706	19775	19810
Median	235	235	235	2200	2235
Max value	74700	74700	2250	74700	74700
P95	50095	50095	1962	64155	64155

*All available concentrations > LOD/LOQ.

Consumption

Considering the EFSA Chronic food consumption statistics of chronic consumption for consumers only, the highest 95th percentile of consumption in a European country is in The Netherlands, with 250 g/day, followed by Germany (150 g/day), Italy (134.5 g/day), Spain (133.6 g/day), Sweden (63.7 g/day) and France (50 g/day). Based on data provided by EU Member States, 400 g of shellfish meat is used by EFSA to assess acute risk of marine biotoxins (EFSA 2010).

MCs intake associated to consumption

Assuming a seafood consumption of 250 g/day and MC concentrations of about 10,000 µg/kg (mean value considering all analytical methods), the intake per person would correspond to about 2.5 mg MC/day for an adult of 60kg bw. When a larger portion of 400 g of seafood is considered, the intake per person would correspond to about 4 mg MC. It has to be highlighted that this calculation has been made with very few data, hence the statistics is not robust enough. Yet, these results highlight a problem that is necessary to be better defined.

This outcome is quite similar even including estimated MC concentrations from DW data, reported in Table 3.7.1.7.

Table 3.7.1.7. Mean, median, maximum and P95 MC concentrations in seafood considering also DW data

	All analytical methods		ELISA methods	Chromatographic methods	
	Conc µg/kg LB	Conc µg/kg UB	Conc µg/kg*	Conc µg/kg LB	Conc µg/kg UB
Mean	6326	6337	618	9892	9911
Median	70	70	328	12	44
Max value	74700	74700	2250	74700	74700
P95	32520	32520	1868	50095	50095

*All available concentrations > LOD/LOQ.

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In this case the intake per person would correspond to about 1.6 mg MC/day for an adult of 60kg bw when a portion of 250g is considered and 2.5 mg MC/day when a larger portion of 400 g of seafood is considered. The intakes are a lower than those calculated only from FW data but still very high.

Considering Crustacean and Mussels separately it is possible to define the worst case scenario as reported in Table 3.7.1.8., although being aware of the limitation due to the paucity of available data on MC occurrence in these organisms.

Table 3.7.1.8 MC concentrations and relative intakes in crustacean and mussels.

	Crustacean (4 data)		Mussels (4 data)	
	MC conc in µg/kg	MC intake (**µg**) considering a consumption of 250 g (or 400g) of crustacean	MC conc in µg/kg	MC intake (**mg**) considering a consumption of 250 g (or 400g) of mussels
Mean	689	172 (275)	19828	5 (8)
Median	217	54 (87)	2271	0.6 (0.9)
Max value	2250	563 (900)	74700	19 (30)
P95	1962	491 (785)	64155	16 (26)

As shown in the table, MC intakes for fresh-water crustacean species are very high (close to the safe acute risk threshold). They are consumed probably sporadically by limited local population, yet their quality is not routinely controlled at least in relation to MC occurrence.
MC intakes by eating mussels (*Mytilus edulis* and *Mytilus galloprovincialis* in the Database, species which are commonly consumed by the general population) are higher than those from crustaceans and are 2 orders of magnitude higher than the safe acute risk threshold. MC presence is not specifically controlled in mussels. Yet the mandatory bioassay to protect consumers' health from exposures to marine algal toxins should be valid also for these cyanotoxins. But these aspects should be further investigated, also in view of the possibility that mussels are grown in aquaculture plant in estuarine and coastal areas, in which cyanobacteria can be present.
In our view, this issue represents probably the main data gap to be adequately filled, in light of the potential risk to consumers (more data and a better interpretation of their implications to human health).

Other edible animals (Game hunting, birds and livestock)

Due to the lack of sufficient MC occurrence data, it is not possible to define a reliable exposure scenario. However, considering the 'fatal attraction' of animals for contaminated water (Codd et al.,1992; Lopez Rodas and Costas, 1999), it cannot be excluded that livestock including dairy cows, beef cattle, sheep, etc. by drinking raw water are exposed to high levels of cyanotoxins. Indeed, the number of case reports of deaths and poisoned domestic animals (mainly dogs) and livestock highlights the relevance of this phenomenon, for which more data are needed. The 'fatal attraction' applies also to mats and cyanobacterial crusts especially related to benthic cyanobacteria, for which data are even more limited.
Although one study indicate the absence of 'accumulation' in meat and milk of cows specifically treated with MC-LR, no information are available on other variants (especially for the more lipophilic one the situation can be markedly different).

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Edible crops

In agreement with EFSA, all laboratory data and studies with non reliable methods have been excluded from the analysis of the exposure scenarios. As a consequence only 1 study could be used related to contamination of chestnut from China. Unfortunately, in this study it is not clear whether the concentrations are expressed on fresh or dry weight, therefore even the relative data cannot be used to define a hypothetical exposure scenario.

Food supplement

It is to highlight that due to the low number of available selected data (12 in total from 4 studies, 6 with ELISA and 6 with chromatographic methods) the statistics is not robust.

Since Food supplements are consumed dry (mainly as capsules) the consideration related to the differences between FW and DW does not apply.

Table 3.7.1.9 MC concentrations in food supplements

	All method of analysis	Only ELISA methods	Only Chromatographic methods
	Conc in µg/kg	Conc in µg/kg	Conc in µg/kg
Mean	2742	3362	1873
Median	241	320	163
Max value	18400	18400	5200
P95	11140	13639	4960

Considering the EFSA Chronic food consumption statistics for consumers only, the highest 95th percentile of consumption for the category "Algae formula (e.g. *Spirulina, Chlorella*)" (at Level3 of the EFSA FoodEx classification system), is 3.75 g/day.

Using this value and considering the mean MC content of 2742 µg/kg (table 3.7.1.9), the daily intake is around 10 µg MC per person, exceeding the daily intake for an adult corresponding to the TDI, but below the subchronic threshold.
Yet, the available data on MC occurrence in food supplements are too limited and more data are needed for an adequate risk assessment. However, 1) by looking at the maximum value (around 6-fold higher than the mean levels), 2) considering that BGAS are consumed following individual of daily dosage, so that the consumption of extremely high daily doses (up to 20 g), has been reported (Dietrich and Hoeger, 2005) and 3) BGAS are specifically addressed to children for the alternative therapy of ADHD, having a lower body weight, and therefore lower threshold for daily tolerable intake, these data are of particular concern.

3.7.2. Cyanotoxins other than Microcystins

The occurrence dataset contains very few data for toxins other than MC that can be used for assessing exposure scenarios. Therefore it was considered that the data set was not robust enough even for making any tentative evaluation. This is identified as a clear data gap.

No occurrence data on LPS and lyngbyatoxins/aplysiatoxins in food are available.

Only 18 data for free BMAA and 18 data for bound BMAA (referred to 8 different fish species from one study) are available and as LPS, no safe threshold reference values have been yet defined.

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Concerning ATX, no data are available for seafood, and only few for fish. Specifically, in 2 studies ATX values are below the limit of detection, in the other 2, a maximum ATX concentration of 36 µg/kg in fish muscle was detected. Assuming a fish portion of 265g/day, the corresponding ATX intake is of about 9.5 µg/day This value is well below the provisional safe chronic reference value of 30 µg/day for an adult of 60kg bw, proposed in this document (Health Based reference values for cyanotoxins). However, being based on few data again, no conclusion can be drawn, and more data are needed.

Four studies investigate ATX occurrence in food supplements, in 3 of them concentrations are below the limit of detection, in the remaining one a maximum concentration of 19 µg/g was reported for epoxyanatoxin-a, an ATX metabolite. The relative intake might be of 19 µg/g x 3.75 g/day= 71.25 µg/day. No toxicological information is available on this ATX metabolite, assuming the same TDI of the parent compound, the safe chronic dose would be exceeded. However, being based on a single study, no conclusion can be drawn, and more data are needed.

In one study PSP up to 0.71 µg/kg has been reported in fish muscle. Assuming the fish consumption of 265g/day, the PSP intake is of 0.2 µg/day for an adult. This intake is well below 30 µg STX equivalents for a 60 kg adult, derived from the ARfD of 0.5 µg STX equivalents/kg bw (Data from other available studies have not been used as they do not specify whether data are expressed as FW or DW). However, being based on a single study, no conclusion can be drawn, and more data are needed.

One study reports a nodularin concentration up to 17.6 µg/kg in fish muscle. The intake is about 5 µg nodularin /day with a fish consumption of 265g/day. Data from other available studies have not been used as they do not specify whether data are expressed as FW or DW.

Nodularin in seafood is reported only in 2 studies in shrimps, with concentrations up to 0.7 µg/kg. In the ANSES dataset concentrations up to 2500 µg/kg are reported for mussel (species not reported) collected during a cyanobacterial bloom in Australia. Yet, it is not clear whether these data refer to FW or DW.

Concerning cylindrospermopsin, only one study is reported for shrimp with values below the detection limit. Only few data are available for CYN in fish. Yet, out of these 3 studies, in 2 it is not clear whether data are expressed as FW or DW, furthermore concentrations are below the limit of detection. In the last study a CYN concentration of 0.24 µg/kg in fish muscle is reported. If the fish portion used for MC is of 265g/day, the intake is of about 0.1 µg/day. This value is well below the chronic reference value of 1.8 µg/day for an adult of 60kg bw proposed in this document. However, being based on a single study, no conclusion can be drawn, and more data are needed. Two studies investigate CYN occurrence in food supplements, with values below the limit of detection.

4. CONCLUSIONS

Although a large number of papers are available on cyanotoxins, the analysis of data indicates that information on this class of compounds is still limited.

Most retrieved papers were on MCs, particularly on one single congener (MC-LR) both for occurrence and toxicity. The paucity of papers addressing cyanotoxins other than MCs (even in the area of their presence in freshwater environments) can leave the mistaken impression they are of minor concern in relation to human health. The low detection frequency may partially reflect the low method sensitivity and the scant availability of certified standards; the need to have analytical validated methods not only for detection of neurotoxins in water but also in complex matrices is absolutely a priority, in order to have reliable data on exposure understanding whether and which further data on their toxicity is needed.

Indeed, one of the major issues is related to the availability of reliable analytical methods to sample preparation and detection of cyanotoxins in complex matrix as food items. While methods have been

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validated for cyanotoxin detection in water, the situation is still problematic when complex matrices and other biological samples are concerned. The main reasons are related to the binding of some cyanotoxins (such as MCs and BMAA) to proteins, making the final recovery unfrequently reported, generally low and highly variable depending on the matrix and also on the variant among the same family of cyanotoxins. Furthermore strong matrix effects affecting the analytical detection, although responsible for significant inaccuracy, are quite often not taken into account.
In addition it is necessary to consider that 1) chromatographic detection methods are very specific for targeted analyses, especially in high resolution, meaning that what is detected is what has been looked for. Therefore the total content of cyanotoxin can be underestimated, although knowing the variants present can give useful information regarding toxicity. On the other hand, biological techniques such as immunological ELISA methods, able to detect analogues of the same family (including unknown ones) provide only semiquantitative information, since the cross-reactivity of the antibody towards the toxin analogues can greatly vary among variants, making any quantification difficult (and no indication about the variants).

On this basis the papers on occurrence included in the inventory were further screened based on a quality score system, taking into account the quality of the analytical method used: those results obtained with an unreliable quantification method (not validated, not considering possible matrix effects, not reporting any recovery information) were not considered for the further elaboration in the exposure scenarios.

Regarding occurrence data, when considering the reliability of the analytical method, only few data remained from field studies expressed as fresh weight (and a small percentage refers to European data). Some laboratory studies on fish and shell-fish are also available, but they generally use high unrealistic exposure conditions and were not considered for further elaboration in the exposure scenarios. In addition, only data expressed as fresh weight were considered as first choice, since, although a conversion factor could be used to translate dry weight into fresh weight data, this extrapolation is affected by a high degree of uncertainty, mainly due to lack of information on the procedure used to obtain dry weight.

The data collected show the occurrence at measurable levels of MC in aquatic edible species from European and non-European countries (North America, South America, African and Asian countries) confirming that accumulation of MC is a global concern. Liver and viscera in fish have been reported to have higher levels than muscle tissue, but no relationship can be found, due to spotted results in different species, that increase variability. In addition the available data seem not to indicate a clear and direct relation between the fish species with different feeding strategies and MCs concentrations measured in their tissues. When the reliability of the analytical method was taken into account, only few studies were available on livestock (relying on a very limited number of animals); one study measured MCs in the muscle of wild birds and game hunting and only one study on crops (on lyophilized samples). No data remain for European countries. Regarding food supplements only 5 studies (2 from EU Countries: range 0.86-5.20 µg MCs/g) passes the quality check for the analytical method used for *A. flos-aquae* products.

The toxins NODs, BMAA, CYN and ATXs have been the most investigated among the group 'toxin other than MC'. Toxin occurrence data in animals or crops through field studies were very limited, when quality criteria were applied. No study was included in the inventory regarding CTX, PlTX, Lyngbya toxin and its congeners. Few studies have been conducted on food supplements derived from *Spirulina* and *Aphanizomenon* only for ATX and CYN showing some variability in their results.

Regarding the toxicological profile, as said, most retrieved papers were on MCs, particularly on one single congener (MC-LR). *In vivo* studies, using a sound and robust study design, methodologies and reporting, useful for the derivation of health based reference values, are unfortunately not many. Among the retrieved papers there are many *in vitro* studies on MeA (mainly on cellular/molecular target related to phosphatase inhibition and oxidative stress induction), sometimes redundant and repetitive. The more recent studies on MC are focused mainly on reproductive toxicity and organ

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toxicity other than liver (e.g. thyroid) in some cases with administration of a single dose (never following any guideline test method), often using the i.p. route of exposure, which is poorly representative of the actual human exposure. Indeed, on the basis of the available data i.p. exposure has marked differences (up to 30 fold) with respect to acute toxicity when compared to the oral route (likely due to kinetic differences).
One limitation to toxicity studies conduction could be the scant availability of the toxins; for this reason studies are often carried out with poorly characterised cyanobacterial extracts (with the toxin presence not always quantified) and therefore not useful for the risk assessment. Indeed, although it could be inferred that this is representative of some actual exposure (i.e. for animal exposed to raw waters), the available data indicate that components other than cyanotoxins are responsible for some toxicity and contribute to increase the toxicity of the extract when compare to 'pure' toxins. This is true for MC variants but also for the other cyanotoxins for which the data base is much more limited.

For some MC variants (a small % with respect to the known ones) it was possible to compare the acute toxicity having some data after i.p. injection: a single aminoacid difference (MC-LR vs MC-RR) can result in a 10-fold difference in the i.p. LD_{50}. A TEF approach has been suggested, but considering the marked difference between the oral and the i.p. route and the possible kinetic differences (involving the active transport mediated by OATP molecule, distribution, metabolism via GSH-conjugation) among other more lipophilic variants, information is likely to be too preliminary to be applied.

For MC-LR a provisional reference value for subchronic exposure (0.4 µg/kg bw/day, or 0.16 µg/kg bw/day depending on the PoD) could be derived, as well as a provisional TDI of 0.04 µg/kg bw/day (as WHO did, to obtain a Guidance Value for drinking water), although with a high degree of uncertainty. The WHO provisional TDI value for MCs, which has hitherto represented the only pragmatic, quantitative approach to the management of risks associated with exposure to this class of cyanotoxins, has some limitations, mainly due to the scarcity of information available not exhaustive in either kinetic or dynamic respects. It can be discussed whether or not an additional safety factor should be included to account for the tumour promoting activity of MC, which on the other hand has a threshold, related to the inhibition of hepatic protein phosphatases. In addition, since the evaluation carried out for one congener MC-LR has been suggested to be extended to all the others, expressing it as MC-LR equivalents, but the validity of extrapolation from the acute toxicity ranking obtained via intraperitoneal injection to other routes of exposure, and most importantly among MC congeners, to chronic toxicity remains to be demonstrated. However, the WHO GV for drinking water is the one to which most of the recommendations/regulation and/or alert values make reference (as described in the appropriate section of this document).

From MC-LR toxicological data it is also possible to estimate safe concentrations with regard to the acute risk following oral exposure: an acute NOAEL of 25 µg/kg body wt, the highest dose at which no hepatic effects are observed can be derived from intraperitoneal injection studies in mice, but again the uncertainty is high, especially considering the steepness of the dose-response curve.

Regarding the other toxins, databases for ATX and CYN on repeated, short and long-term oral toxicity have not been deemed adequate by WHO to derive any TDI. A tentative health based value derivation has been proposed for ATX (no 'significant' effect observed at the highest doses tested) and CYN, and used for setting limits or guidelines in some Countries, but the levels of uncertainty is even higher than for MC, especially related to CYN potential, but still controversial, genotoxicity, associated to its metabolites (not identified so far). Again data are needed to confirm or modify these values.

For those toxins already evaluated by EFSA (EFSA Journal, 2009a,b, 2010), some new data are available which could give indications for an update. However, data are still limited and the derivation of health based values would still be affected by a high degree of uncertainty.

The paper by Munday et al. (2013) on STX tackled the problem of the use of the Toxicity Equivalence Factors (TEFs), based on the MBA method, currently applied following the EFSA opinion (2009). When oral acute toxicity was considered, the obtained TEF values were different from the ones used by

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EFSA relevant particularly in the case of NeoSTX more toxic than STX (assumed as the more toxic based on acute i.p. toxicity). This was confirmed also by the no acute effect level estimated after oral administration.

For PlTX the ArfD is at present derived after sublingual administration, according to the EFSA opinion (2009): data after oral administration now available basically confirmed the acute toxicity for PlTX and its analogue 42-OH-PlTX. However, it could be interesting to consider a NOAEL = 3 mg/kg/day derived from a short term repeated toxicity study in mice, which can be representative of human exposure scenarios i.e. through repeated consumption of contaminated seafood at least for a short period of time.

The Extensive Literature Search results evidenced that environment plays a very complex role in the toxicity of the bloom, in terms of fitness of the potentially toxic genotype, regulation of MC production, or MC quota. Regarding the environmental factors possibly affecting the toxicity of a bloom, most of the studies were dedicated to MC, and among them > 80% of paper deals with *Microcystis* sp. Both from field and laboratory studies different environmental parameters (nutrient levels, particularly P and N; temperature, CO_2 levels, light, iron, inorganic carbon) were considered with respect to MC production and/or *mcy* expression), but results are quite often controversial, mainly due to the fact that data are obtained in different experimental conditions, using different methods of measuring parameters and are hardly comparable. It seems clear that not-limiting nutrient concentrations are the basis for an abundant bloom, which is also the assumption for a risky situation, even if the thresholds can be different for the various species. Less clear is the nutrient role in the toxicity of the bloom.

The number of studies on other toxins are much less than the ones for MCs. The organisms producing toxins considered in the available studies are diverse, as well as the environmental conditions and parameters: data are therefore sparse and not comparable, raising more questions than answers.

Toxicological interactions can be expected among cyanotoxins and between cyanotoxins and other contaminants, based on some information on the MeA, besides being plausible due to the concurrent presence in the environment and likely in food items. Despite this, very few papers were selected, regarding possible mixture toxicity between cyanotoxins and other chemicals. However, since in many cases health based reference values and the toxicological profile of the single toxins are not sufficiently elucidated, the evaluation of possible mixture toxicity is premature and can be, if any, only qualitative.

Based on the occurrence database, exposure scenarios are tentatively described for the EFSA categories of fish and other seafood, BGAS and crops; consumption of fish/shellfish (especially regarding freshwater organisms) was considered as reported in the EFSA consumption database, as well as food supplement consumption. Although the database including reliable results from field studies is very limited, some consideration can be tentatively derived.

Some studies report also MC concentrations in fish liver and viscera, with values generally much higher than those in muscle tissues. Yet, they are not normally consumed according to European diet styles and for this reason they were not included in the exposure scenarios. On the other hand, we cannot exclude that some specific considerations might be done at local level in relation to this potential exposure (e.g. some specific groups of immigrants).

Assuming the highest fish consumption (265g/day) and MC mean concentrations in fish edible tissue of 187-204 µg/kg, the intake for an adult person of 60 kg bw would be about 50-54 µg MC/day. These values are well above that of 2.4 µg/day per adult (the chronic safe threshold derived from the TDI of 0.04 µg/day/kg bw, defined in the WHO guidelines (2003, 2004)). It can be discussed whether for the general population a chronic daily consumption is a realistic exposure scenario, or this kind of exposure could be limited to local populations (e.g. fishermen and their families). However, the estimated ingestion also exceeds the subchronic reference value (24 µg/day, obtained using the same PoD as for the TDI, or around 8 µg/day with the more conservative value), covering the consumption of contaminated fish for a limited period of time. This is a more realistic scenario also considering the

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seasonality of cyanobacterial blooming. In addition the maximum value (obtained with a chromatographic analytical method) is more than 1 order of magnitude higher than the mean value. Considering that MCs occur mainly in freshwaters fish species, focus has been given to the *Cyprinidae* family, accounting for 68% of the available data, for which specific data about consumption are available on the EFSA data-base for food consumption. Again, the corresponding MC intakes are above the safe chronic risk threshold and close to the subchronic one. However it should be underlined that information on MC production by cyanobacteria genera in estuarine or marine waters –although potentially relevant- are not available at present.

Even when considering shellfish consumption, the daily intake per person would correspond to about 2.5 mg MC/day for an adult of 60kg bw, which is an extremely high level. Trying to distinguish between molluscs and crustaceans, the former represents by far the worst case (2 orders of magnitude higher than the safe acute risk threshold). It has to be highlighted that this calculation has been made with very few data, hence the statistics is not robust enough. Yet, these results highlight a problem that is necessary to better define.
Indeed, freshwater crustacean are likely to be sporadically consumed by limited local population, yet their quality is not routinely controlled, at least in relation to MC occurrence.

Mytilus edulis and *Mytilus galloprovincialis* on which data are available are species commonly consumed by the general population: however, MC presence is not specifically controlled in mussels. Yet, the mandatory mouse bioassay to protect consumers' health from exposures to marine algal toxins should cover also the presence of these cyanotoxins. These aspects should be further investigated, also in view of the possibility that mussels are grown in aquaculture plant in estuarine and coastal areas, in which cyanobacteria can be present.
In our view, this issue represents probably the main data gap to be adequately filled, in light of the potential risk to consumers (more data and a better interpretation of their implications to human health). This consideration is further supported by laboratory studies, clearly indicating the possibility to accumulate cyanotoxins at high levels in fish and shell-fish.

A special consideration has to be given to BGAS, for which exposure scenarios at risk have been identified for MC contamination, although again associated to a data-poor situation.
For cyanotoxins other than MCs, the knowledge about occurrence is even worse. Due to the large increase in food supplements market and the large amount that people, including children, can ingest, more investigation on occurrence of cyanotoxins is needed.

For the other food items (e.g. crops, game hunting, meat, eggs or dairy products) information was so scant that it was not considered appropriate to describe any exposure scenario. Further research is needed before drawing any conclusion, and data cannot be limited to MC-LR regarding also the possible transfer of more lipophilic MCs to milk or meat, especially considering the 'fatal attraction' of animals for contaminated water. On this basis, it cannot be excluded that livestock including dairy cows, beef cattle, sheep, are exposed to high levels of cyanotoxins by drinking raw water. Indeed, the number of case reports of deaths and poisoned domestic animals (mainly dogs) and livestock highlights the relevance of this phenomenon, for which more data are needed. The 'fatal attraction' applies also to mats and cyanobacterial crusts especially related to benthic cyanobacteria, for which data are even more limited.
Regarding crops, as the contact with toxins is mostly due to their presence in the irrigation water, if the water used for this purpose is controlled concerning the key toxins showing bioaccumulation in laboratory studies, this would not be a main issue, but in the absence of this control, more data are needed.

5. RECOMMENDATIONS

Analysis of the available data retrieved after the Extensive Literature Search indicates that additional efforts should be put into elucidating the levels of human exposure to cyanotoxins under different

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scenarios, not only related to drinking water, as well as in the identification of repeated-dose toxicity for cyanotoxins other than MC-LR. This is necessary for the derivation of GVs and regulatory limits as well as for the characterization of further emerging cyanotoxins, and interactions between them, other toxicants and microbial pathogens.

As indicated in the Conclusions section, one of the major issues is related to the availability of reliable analytical methods to sample preparation and detection of cyanotoxins in complex matrix as food items and other biological samples, since methods have been validated for cyanotoxin detection in water. Based on all these elements, when handling occurrence data as part of risk assessment, a higher weight should be given to those obtained with methods taking into account all the toxin fractions (free and bound), incorporating cleanup steps assessed for recovery and for which characterization/validation data are readily available for the considered toxin/matrix couple.

The data base on occurrence on which preliminary considerations about the possible risks for consumers were derived is extremely limited and the production of more data and various food items (including food supplements) is highly recommended and should not be limited to MC-LR.

Regarding the toxicological profile, still many data gaps are present, even in the case of MC, the most studied group among cyanotoxins. It is recommended to use administration regimen which are representative of the actual human situation (e.g. oral repeated exposure), rather than using the i.p. injection, also in view of the marked kinetic differences between the two routes. Furthermore it is recommended to follow guideline test methods and to provide appropriate reporting, avoiding single dose studies, focused on few parameters on specific organ toxicity end-points, using well characterised test items. Indeed, to overcome the limited availability of pure toxins, in some cases toxicity studies are carried out with uncharacterised cyanobacterial extracts (without any toxin quantification), and therefore not useful for the risk assessment.

Therefore, recommendation for more appropriate study design in toxicological studies is warranted.

In this chapter the identified data gaps have been reported in a schematic way.

5.1. Data gaps on occurrence

MC

More data related to MC occurrence in food (irrespective on the food item) detected with reliable analytical method and extraction procedures are needed on:

- Different variants (not only MC-LR)
- the effect of cooking on the concentration of MC
- the proportion of free vs bound microcystins in the tissues
- the bioavailability of bound microcystins
- the occurrence of MC in edible animals, crops and food supplements
- the occurrence of MC in milk and meat from livestock

Toxins other than MC

For all toxins, there is the need to quantify them in food, feed and food supplements by using reliable and validated analytical methods, able to distinguish among variants. Other specific points:

- The proportion of free vs bound toxins in the tissues is not known and should be further investigated. Indeed, as for MC, some toxins have been reported to bind to proteins. In most cases, only the values of the free toxin in the tissue are given. Therefore, the information on the % of bound toxin is rarely available.

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- To feed the data base on occurrence of those toxins in different food items in relation with blooms presence. It appears that the values can vary largely among tissues, individuals, site and time of sampling depending on the presence of cyanobacterial blooms and the variability in the production of toxins.
- The effect of cooking has been investigated only in few cases.
- Irrespective of the toxins, data on edible crops are scant and have been mainly obtained from laboratory studies.
- For STXs, additional data are required to get a larger dataset on freshwater species (shellfish, crustacean, fish), since an indication for bioaccumulation is present (as for PSP from marine species).
- Other toxins are likely produced by some strains of cyanobacteria such as *Cylindrospermopsis raciborskii* but which have not been identified and research of new toxin should be encouraged.

5.2. Data gaps on analytical methods

- Need for chemical standards for cyanotoxins/variants/metabolites other than MC-LR
- Need of validated methodologies for matrix other than water (considering matrix effects and reporting % of recovery).
- Increased availability and standardization of rapid, transferrable and cost-effective analytical methods for different cyanotoxins to be used in monitoring programmes.

5.3. Data gaps on hazard identification and characterization

Recommendation for a more appropriate study design in toxicological studies is warranted.

- Characterization of the toxicological profile of different MC congeners not limited to acute exposure, but related to short-term and chronic effects (looking at other effects in addition to hepatotoxicity and using the oral route rather than the i.p. injection). This would mean to have an adequate quantity of 'pure' toxin available at a reasonable cost.
- Possibility to develop a TEF approach (on the basis of data collected according to the previous bullet point) to evaluate the toxicity of mixtures of congeners (e.g. for MCs and STXs), often present in natural blooms.
- Characterization of the toxicological profile of other cyanotoxins (see above for common issues with MC)
- Characterization of the kinetic profiles (ADME parameters) of different cyanotoxins to carry out route-to-route extrapolation; understanding abiotic/biotic detoxication/bioactivation capability especially in humans, to identify possible susceptible individuals.
- Characterization of cyanotoxins bioavailability (e.g. bound versus unbound fraction) with the aim to understand the relationship (and possibly model it).
- Characterization of the kinetics of bioaccumulation/biomagnification in food and feed (depending on the cyanotoxin source) with the aim to understand the relationship between cyanotoxin in the bloom and food contamination (and possibly model it).
- Characterization of the mechanisms of action of different cyanotoxins to estimate the occurrence of possible additive or synergistic effects and interactions with other toxins and hazardous chemicals.
- Possible mixture toxicity to be further investigated.
- Identification of biomarker of exposure, effects and -if needed- susceptibility to carry out appropriate biomonitoring studies in exposed populations.

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5.4. Data gaps on environmental factors

- More efforts should be done to understand the ecological role of cyanotoxins and to develop models able to predict their production (in terms of community shift towards dominance of toxic genotype; production of more toxic cyanotoxin variants; increase in cyanotoxin production rate) in the future scenarios related to climate changes (expected to increase the diffusion and the abundance of cyanobacteria). Temperature increase and longer stratification periods, flooding of estuarine environment reducing salinity and carrying inocula of cyanobacteria triggering blooms, increase of nutrients due to droughts or to floods towards coastal environments, all have been shown to favour spatial and temporal distribution of cyanobacteria blooms (and some models appeared to forecast these phenomena), but little information is available on their effects on the toxicity of the blooms.
- New monitoring programs including cyanobacteria should be developed for environments like estuaries and costal zones, where aquaculture plants are located, and where cyanobacteria and other marine organisms producing the same toxins (e.g. *O. ovata* and or dinoflagellates for PlTX or CTX, respectively) can be concurrently present.
- More laboratory experiments are needed on model organisms to eliminate the genetic variability of the population and to look at the biochemical pathways and mechanisms of production of MC; on the other hand, mesocosm experiments which include the high genetic variability of the natural population should be performed, to have a more realistic response of cyanobacteria to the manipulation of environmental factors. All parameters should be measured at the same time, to identify the critical step in MC production.
- More experiments on species different from *Microcystis* should be carried out, because it is difficult to extrapolate results from one strain of *Microcystis* to a natural population; even more difficult is to extrapolate results from one strain of *Microcystis* to morphologically and physiologically different species.
- Positive and negative interactions between heterotrophic bacteria, including possible pathogenic taxa, and cyanobacteria are very important in defining the composition and dynamics of microbial communities in nature and may represent an additional threat for human health related to cyanobacteria blooms.

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Toxicological profile

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Abbreviations

ACh	Acetylcholine
AChE	Acetylcholinesterase
ADME	Absorption, Distribution, Metabolism, and Excretion
AF	Allocation Factor
AL	Alert Level
ALP	Alkaline Phosphatase
ALS/PDC	Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia Complex
ARfD	Acute Reference Dose
AST	Aspartate Aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
ATX	Anatoxin-a
ATX-s	Anatoxin-a(S)
AUC	Area Under the Curve
BBB	Blood Brain Barrier
BGAS	Blue-Green Algae Supplements
BMAA	β-N-methylamino-L-alanine
CE	Capillary Electrophoresis
CF	Correction Factor
CFP	Ciguatera Fish Poisoning
CFSAN	FDA Center for Food Safety and Applied Nutrition
CIPPIA	Colorimetric Immune Protein Phosphatase Inhibition Assay
CTX	Ciguatoxin
CVM	Center for Veterinary Medicine
CWA	Clean Water Act
CYN	Cylindrospermopsin
DAB	DL-2,4-diaminobutyric acid dihydrochloride
DAT	Debromoaplysiatoxin
DSHEA	Dietary Supplement Health and Education Act
DSI-EC	Dietary Supplements Information Expert Committee
DW	Dry Weight
ELISA	Enzyme-Linked Immunosorbent Assay
ELS	Extensive Literature Search
FD&C Act	Federal Food, Drug and Cosmetic Act

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FLD, FL	Fluorescence Detection
FW	Fresh Weight
GC	Gas Chromatography
GRAS	Generally Recognized As Safe
GSH	Glutathione
GST	Glutathione S-Transferase
GTX	Gonyautoxin
GV	Guidance Value
HA	Health Advisory
HBGV	Health-Based Guidance Value
HILIC	Hydrophilic Interaction Liquid Chromatography
HLB	Hydrophilic-Lipophilic Balance
HPLC	High Performance Liquid Chromatography
HPLC-FD	High-Performance Liquid Chromatography with Fluorescence Detection
HRMS	High Resolution/Accurate Mass Spectrometer
HTX	Homoanatoxin-a
i.p.	intraperitoneal
i.v.	intravenous
IARC	International Agency for Research on Cancer
IM	Ion Mobility
LA	Lyngbyatoxin A
LAL	Limulus Amœbocyte Lysate
LC	Liquid Chromatography
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LC-UV	Liquid Chromatography-UV
LD50	Lethal Dose 50
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit Of Detection
LOQ	Limit Of Quantification
LPS	Lipopolysaccharide
MAC	Maximum Acceptable Concentration
MAV	Maximum Allowable Value
MBA	Mouse Bioassay
MC	Microcystin
MeA	Mechanism of Action
MoA	Mode of Action

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MS	Mass Spectrometry
NMR	Nuclear magnetic resonance
NOAEL	No Observed Adverse Effect Level
NOD	Nodularin
OATP	Organic Anion Transporting Polypeptide
OST-D	Ostreocin-D
OVTX	Ovatoxin
PlTX	Palytoxin
p.o.	per os
PoD	Point of Departure
PP	Protein Phosphatase
PPIA	Protein Phosphatase Inhibition Assay
PSP	Paralytic shellfish poisoning
SPE	Solid phase extraction
SPME	Solid Phase Micro Extraction
STX	Saxitoxin
t½	Half Life
TDI	Tolerable Daily Intake
TEF	Toxicity Equivalent Factor
TGA	Therapeutic Goods Administration
UF	Uncertainty Factor
UPLC/MS	Ultra-performance liquid chromatography/Mass Spectrometry
US EPA	US Environmental Protection Agency
USP	United States Pharmacopeial Convention
USP–NF	United States Pharmacopeia and National Formulary
WHO	World Health Organization

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Appendixes

This Final Report is supplemented by 3 Appendices:

1) **Appendix A: Information source forms**, which is reported below.
2) **Appendix B: EndNote™ Library**. It corresponds to the full list of references in an EndNote™ Library, provided as a set of files corresponding to different cyanotoxins; within each file the papers have been further subdivided depending on the matrix in the case of occurrence, and on the species (i.e. fish or mammals) or the toxicity endpoint for the toxicological profile definition; separate EndNote™ files are provided for the environmental factors affecting cyanobacterial toxicity and for the mixture toxicity. It can be downloaded from the EFSA website together with this report.
3) **Appendix C: Data Inventory.** It is the data inventory (in tabular format, provided as a collection of MS Excel spreadsheets, a subset for each cyanotoxin, further subdivided depending on the matrix in the case of occurrence, and in vitro, in vivo and epidemiological studies for hazard identification and characterization) containing the data extracted from the relevant papers. It can be can be downloaded from the EFSA website in a Microsoft Excel format together with this report.

Appendix A – Information Source Forms

This Appendix included the information source forms, reporting the queries used in the extensive literature search on the different databases (for the different tasks: occurrence in food, analytical methods, toxicity, environmental factors affecting toxicity and mixture toxicity involving cyanotoxins), the detailed information on the number of initially retrieved papers, the included/excluded papers during the screening phases, and of those papers from which data have been taken and included in the inventory.

A.1 Microcystins occurrence in food

Table 1: Information source form for microcystins occurrence in food

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Nathalie ARNICH
STUDY QUESTION	**Objective 1: cyanobacteria toxins occurrence in food** **Search 1: Occurrence** **Part 1: Microcystins**
Information source	**Scopus** (performed by ANSES)
Date accessed	21 April 2015
Step 1 (broad search): Search terms	TITLE-ABS-KEY (microcystin* OR mc-rr OR mc-lr OR mc-yr OR mc-ar OR mc-lf OR mc-lw OR mc-fr OR mc-delr OR mc-derr OR mc-delf OR mc-1030) AND (presence* OR occur* OR *concentration* OR level* OR bloom* OR *accumulation* OR field* OR laboratory*)

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Number of records retrieved step 1	3429
Step 2 (focused search): Search terms	TITLE-ABS-KEY(microcystin*ORmc-rrORmc-lrORmc-yrORmc-arORmc-lfORmc-lwORmc-frORmc-delrORmc-derrORmc-delfORmc-1030)AND(presence*ORoccur*ORconcentration*ORlevel*ORbloom*ORaccumulation*ORfield*ORlaborator*ORfood*OR"food supplement*"ORfish*ORshellfish*ORmilk*OR"dairy product*"ORcrustacean*ORmussel*ORmollusk*ORaquatic*ORtrout*ORprawn*ORseafood*ORseabird*OReider*ORduck*ORmeat*ORvegetable*ORfruit*ORplant*OR"trophic transfer*"ORfeeding*)
Number of records retrieved step 2	3645
Number of records retrieved step 1 + step 2	7074
Duplicates	3435
Number of records retrieved	3639
Information source	**PubMed** (performed by ANSES)
Date accessed	21 April 2015
Step 1: Search terms	TITLE-ABS (microcystin* OR mc-rr OR mc-lr OR mc-yr OR mc-ar OR mc-lf OR mc-lw OR mc-fr OR mc-delr OR mc-derr OR mc-delf OR mc-1030) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)[15]
Number of records retrieved step 1	1992
Step 2: Search terms	TITLE-ABS (microcystin* OR mc-rr OR mc-lr OR mc-yr OR mc-ar OR mc-lf OR mc-lw OR mc-fr OR mc-delr OR mc-derr OR mc-delf OR mc-1030) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved step 2	2088
Number of records retrieved step 1 + step 2	4080
Duplicates	1988

[15] (((((((((microcystin*[Title/Abstract] AND occurrence*[Title/Abstract])) OR (microcystin*[Title/Abstract] AND concentration*[Title/Abstract])) OR (microcystin*[Title/Abstract] AND level*[Title/Abstract])) OR (microcystin*[Title/Abstract] AND bloom*[Title/Abstract])) OR (microcystin* AND accumulation*[Title/Abstract])) OR(microcystin* AND biomagnification*[Title/Abstract])) OR (microcystin* AND field*[Title/Abstract])) OR (microcystin* AND laboratory*[Title/Abstract]))

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Number of records retrieved		2092
		Scopus + PubMed
Number of records retrieved		5731
Duplicates		1289
Number of records for primary screening		**4442**
Primary screening (exclusion: article in Chinese, fish embryo/larvae, Daphnia, zooplankton)		achieved on 8 May 2015
Primary screening	crops/vegetables/macrophytes	**87**
	animals	**368**
	food supplements	**20**
	monitoring/regulation/health alert levels	45
	data gaps	5
	exposure scenario	11
Information source		**Web of Science** (performed by ISS)
Date accessed		28 April 2015
Step 1 (broad search): Search terms		TOPIC ((microcystin* OR mc-rr OR mc-lr OR mc-yr OR mc-ar OR mc-lf OR mc-lw OR mc-fr OR mc-delr OR mc-derr OR mc-delf OR mc-1030) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*))
Number of records retrieved step 1		3646
Step 2 (focused search): Search terms		TOPIC ((microcystin* OR mc-rr OR mc-lr OR mc-yr OR mc-ar OR mc-lf OR mc-lw OR mc-fr OR mc-delr OR mc-derr OR mc-delf OR mc-1030) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*))
Number of records retrieved step 2		3856
Information source		**Agricola** (performed by ISS)
Date accessed		24 April 2015
Step 1 (broad search): Search terms		(microcystin* OR mc-rr OR mc-lr OR mc-yr OR mc-ar OR mc-lf OR mc-lw OR mc-fr OR mc-delr OR mc-derr OR mc-delf OR mc-1030)

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	AND (presence* OR occur* OR *concentration* OR level* OR bloom* OR *accumulation* OR field* OR laboratory*)
Number of records retrieved	554
Information source	**Food Science Source** (performed by ISS)
Date accessed	28 April 2015
Step 1 (broad search): Search terms	TITLE-ABS-KEY (microcystin* OR mc-rr OR mc-lr OR mc-yr OR mc-ar OR mc-lf OR mc-lw OR mc-fr OR mc-delr OR mc-derr OR mc-delf OR mc-1030) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved step 1	382
Step 2 (focused search): Search terms	TITLE-ABS-KEY (microcystin* OR mc-rr OR mc-lr OR mc-yr OR mc-ar OR mc-lf OR mc-lw OR mc-fr OR mc-delr OR mc-derr OR mc-delf OR mc-1030) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved step 2	403
Number of records retrieved from all databases, duplicates removed	**6 882**
Number of records after primary screening	

	edible animals	**291**	
		high priority	**90**
		medium priority	32
		low priority	169
	non-edible animals	59	
	edible crops	**66**	
		high priority	**17**
		low priority	49
	non-edible crops	26	
	food supplements	**23**	
	fish and other animals by injection	56	
	dead animals	35	

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Secondary screening		
Number of records included in the inventory		
	food supplements	**10**
	edible crops	**15**
	edible animals	**78**
Number of papers **not included** in the inventory but cited in the text		
	food supplements	0
	edible crops	3
	edible animals	8
Number of records **not meeting criteria** for inclusion		
	food supplements	5
	edible crops	1
	edible animals	39
Number of **review papers** not included in the inventory		
	food supplements	8
	edible crops	1
	edible animals	7
Number of missing papers		7

Overall, 175 full papers have been reviewed for possible inclusion in the inventory. The data of 103 papers have been included in the inventory.

Number of records **not reviewed** for possible inclusion		
edible animals	high priority	**completed**
	medium priority	**completed**
	low priority	159 (10 reviewed)
edible crops	high priority	**completed**
	low priority	45

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A.2 CYANOTOXINS OTHER THAN MICROCYSTINS: OCCURRENCE IN FOOD

Table 2: Information source form for cyanotoxins other than microcystins: occurrence in food

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Ateliana SIALEHAAMOA
STUDY QUESTION	Objective 1: Search 1: Occurrence **Groups: Anatoxin-a/Homoanatoxin-a/ Anatoxin-a(S)**
Information source	**Scopus** (performed by ANSES)
Date accessed	21 April 2015
Search terms for STEP1 (broad search)	TITLE-ABS-KEY (*anatoxin*) AND (presence* OR occur* OR *concentration* OR *accumulation* OR bloom* OR level* OR field* OR laboratory*))
Number of records retrieved STEP1	618
Search terms for STEP2 (focused search)	TITLE-ABS-KEY(*anatoxin*) AND (presence* OR occur* OR *concentration* OR *accumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR "food* supplement*" OR fish* OR shellfish* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR milk* OR "dairy product*" OR vegetable* OR fruit* OR plant* OR "trophic* transfer*" OR feeding*)
Number of records retrieved STEP2	711(- 4 duplicates)= **707**
Information source	**Pubmed** (performed by ANSES)
Date accessed	21 April 2015
Search terms for STEP1 (broad search)	TITLE-ABS (anatoxin* OR Homoanatoxin*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	303
Search terms for STEP2 (focused search)	TITLE-ABS (anatoxin* OR Homoanatoxin*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2	**314**
Information source	**Scopus + Pubmed**
Number of records retrieved	**1021**

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Duplicates		**292**
Number of records retrieved for primary Screening		**729**
Primary screening		
	In water (exclusion criteria)	78
	In edible matrix	63
Secondary screening (included in the inventory)		Started on 11 May 2015
Information source:		**Web of science** (performed by ISS)
Date accessed:		28 April 2015
Step 1 (broad search): Search terms		TOPIC (anatoxin* OR Homoanatoxin*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1		**578**
Step 2 (focused search): Search terms		TOPIC (anatoxin* OR Homoanatoxin*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2		**624**
Information source		**Food Source Science** (performed by ISS)
Date accessed		28 April 2015
Step 1 (broad search): Search terms		TITLE-ABS-KEY (anatoxin* OR Homoanatoxin*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1		40
Step 2 (focused search): Search terms		TITLE-ABS-KEY (anatoxin* OR Homoanatoxin*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2		**42**
Information source		**Web of science (ISS) + Food Source Science (ISS)**
Number of records retrieved STEP 2		**666**
Duplicates		**36**
Number of records retrieved		**630**
Duplicates with Scopus + Pubmed		**443**

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Number of records retrieved for primary screening	**187**
EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Ateliana SIALEHAAMOA
STUDY QUESTION	Objective 1: Search 1: Occurrence **Groups: Cylindrospermopsins**
Information source	**Scopus** (performed by ANSES)
Date accessed	22 April 2015
Search terms for STEP1 (broad search)	TITLE-ABS-KEY (cylindrospermopsin*) AND (presence* OR occur* OR *concentration* OR *accumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	424
Search terms for STEP2 (focused search)	TITLE-ABS-KEY (cylindrospermopsin*) AND (presence* OR occur* OR *concentration* OR *accumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR "food* supplement*" OR fish* OR shellfish* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR milk* OR "dairy product*" OR vegetable* OR fruit* OR plant* OR "trophic* transfer*" OR feeding*)
Number of records retrieved STEP2	449 (-2 duplicates) **= 447**
Information source	**Pubmed** (performed by ANSES)
Date accessed	22 April 2015
Search terms for STEP1 (broad search)	TITLE-ABS (cylindrospermopsin*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	256
Search terms for STEP2 (focused search)	TITLE-ABS (cylindrospermopsin*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2	**267**
Information source:	**Scopus + Pubmed**
Number of records retrieved	**714**
DUPLICATES:	**365**
Number of records retrieved	**349**
Primary screening	

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	In water (exclusion criteria)	78
	In edible matrix	46
Secondary screening (included in the inventory)		Started on 11 May 2015
Information source		**Web of science** (performed by ISS)
Date accessed		28 April 2015
Search terms for STEP1 (broad search)		TOPIC (cylindrospermopsin*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1		425
Search terms for STEP2 (focused search)		TOPIC (cylindrospermopsin*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2		**443**
Information source		**Food Source Science** (performed by ISS)
Date accessed		28 April 2015
Search terms for STEP1 (broad search)		TITLE-ABS-KEY (cylindrospermopsin*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1		50
Search terms for STEP2 (focused search)		TITLE-ABS-KEY (cylindrospermopsin*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2		**54**
Information source		**Web of science (ISS) + Food Source Science (ISS)**
Number of records retrieved STEP 2		**497**
Duplicates		**55**
Number of records retrieved (duplicates removed)		**442**
Duplicates with Scopus +Pubmed		**303**
Number of records retrieved		**139**
EFSA SYSTEMATIC REVIEW		Cyanobacteria toxins in food
EFSA REFERENCE		OC/EFSA/SCER/2014/04

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INFORMATION SCIENTIST	Ateliana SIALEHAAMOA
STUDY QUESTION	Objective 1: Search 1: Occurrence **Groups: Nodularin**
Information source	**Scopus** (performed by ANSES)
Date accessed	22 April 2015
Search terms for STEP1 (broad search)	TITLE-ABS-KEY (nodularin*) AND (presence* OR occur* OR *concentration* OR *accumulation* OR bloom* OR level* OR field* OR laboratory*))
Number of records retrieved STEP1	381
Search terms for STEP2 (focused search)	TITLE-ABS-KEY(nodularin*) AND (presence* OR occur* OR *concentration* OR *accumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR "food* supplement*" OR fish* OR shellfish* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR milk* OR "dairy product*" OR vegetable* OR fruit* OR plant* OR "trophic* transfer*" OR feeding*)
Number of records retrieved STEP2	**405 (-1 duplicate)= 404**
Information source	**Pubmed** (performed by ANSES)
Date accessed	22 April 2015
Search terms for STEP1 (broad search)	TITLE-ABS (nodularin *) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	197
Search terms for STEP2 (focused search)	TITLE-ABS (nodularin *) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2	**205**
Information source	**Scopus + Pubmed**
Number of records retrieved	**609**
Duplicates	**200**
Number of records retrieved for primary screening	**409**
Primary screening	
In water (exclusion criteria)	27
In edible matrix	81
Secondary screening (included in the inventory)	Started on 11 May 2015
Information source	**Web of science** (performed by ISS)

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Date accessed	28 April 2015
Search terms for STEP1 (broad search)	TOPIC (nodularin *) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	433
Search terms for STEP2 (focused search)	TOPIC (nodularin *) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2	**459**
Information source	**Food Source Science** (performed by ISS)
Date accessed	28 April 2015
Search terms for STEP1 (broad search)	TITLE-ABS-KEY (nodularin *) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	28
Search terms for STEP2 (focused search)	TITLE-ABS-KEY (nodularin *) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2	**28**
Information source	**Web of science (ISS) + Food Source Science (ISS)**
Number of records retrieved STEP 2	**487**
Duplicates	**24**
Number of records retrieved	**463**
Duplicates with Scopus+Pubmed	**322**
Number of records retrieved for primary screening	**141**

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Ateliana SIALEHAAMOA
STUDY QUESTION	Objective 1: Search 1: Occurrence Groups: **CYANO KEYWORDS**

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Information source	**Scopus** (performed by ANSES)
Date accessed	22 April 2015
Search terms for STEP1 (broad search)	TITLE-ABS-KEY ((cyanobacter* AND toxin*) OR cyanotoxin* OR ("blue-green alga*" AND toxin*) OR ("blue alga*" AND toxin*)) AND (presence* OR occur* OR *concentration* OR *accumulation* OR bloom* OR level* OR field* OR laboratory*))
Number of records retrieved STEP1	3197
Search terms for STEP2 (focused search)	TITLE-ABS-KEY ((cyanobacter* AND toxin*) OR cyanotoxin* OR ("blue-green alga*" AND toxin*) OR ("blue alga*" AND toxin*)) AND (presence* OR occur* OR *concentration* OR *accumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR "food* supplement*" OR fish* OR shellfish* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR milk* OR "dairy product*" OR vegetable* OR fruit* OR plant* OR "trophic* transfer*" OR feeding*))
Number of records retrieved STEP2	3458 (-27 duplicates)= **3431**
Information source	**Pubmed** (performed by ANSES)
Date accessed	22 April 2015
Search terms for STEP1 (broad search)	TITLE-ABS ((cyanobacter* AND toxin*) OR cyanotoxin* OR (blue-green alga* AND toxin*) OR (blue alga* AND toxin*)) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	1410
Search terms for STEP2 (focused search)	TITLE-ABS ((cyanobacter* AND toxin*) OR cyanotoxin* OR (blue-green alga* AND toxin*) OR (blue alga* AND toxin*)) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2	**1501**
Information source	**Scopus + Pubmed**
Number of records retrieved	**4932**
Duplicates	**1454**
Number of records retrieved for primary Screening	**3479**
Primary screening	
In water (exclusion criteria)	333
In edible matrix	521
Secondary screening (included in the inventory)	Started on 11 May 2015

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Information source	**Web of science** (performed by ISS)
Date accessed	28 April 2015
Step 1 (broad search): Search terms	TOPIC ((cyanobacter* AND toxin*) OR cyanotoxin* OR (blue-green alga* AND toxin*) OR (blue alga* AND toxin*)) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	3424
Step 2 (focused search): Search terms	TOPIC ((cyanobacter* AND toxin*) OR cyanotoxin* OR (blue-green alga* AND toxin*) OR (blue alga* AND toxin*)) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumula-tion* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2	**3689**
Information source	**Food Source Science** (performed by ISS)
Date accessed	28 April 2015
Step 1 (broad search): Search terms	TITLE-ABS-KEY ((cyanobacter* AND toxin*) OR cyanotoxin* OR (blue-green alga* AND toxin*) OR (blue alga* AND toxin*)) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	309
Step 2 (focused search): Search terms	TITLE-ABS-KEY ((cyanobacter* AND toxin*) OR cyanotoxin* OR (blue-green alga* AND toxin*) OR (blue alga* AND toxin*)) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2	**344**
Information source	**Web of science (ISS) + Food Source Science (ISS)**
Number of records retrieved STEP 2	**4033**
Duplicates	**319**
Number of records retrieved (duplicates removed)	**3714**
Duplicates with Scopus+ Pubmed (duplicates with the records retrieved for each specific toxin not	**2817**

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considered at this stage)	
Number of records retrieved for primary screening	**897**
EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Ateliana SIALEHAAMOA
STUDY QUESTION	Objective 1: Search 1: Occurrence Groups: **BMAA**
Information source	**Scopus** (performed by ANSES)
Date accessed	22 April 2015
Search terms for STEP1 (broad search)	TITLE-ABS-KEY (β-N-Methylamino-l-alanine* OR BMAA*) AND (presence* OR occur* OR *concentration* OR *accumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	220
Search terms for STEP2 (focused search)	TITLE-ABS-KEY (β-N-Methylamino-l-alanine* OR BMAA*) AND (presence* OR occur* OR *concentration* OR *accumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR "food* supplement*" OR fish* OR shellfish* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR milk* OR "dairy product*" OR vegetable* OR fruit* OR plant* OR "trophic* transfer*" OR feeding*)
Number of records retrieved STEP2	255 (-6 duplicates)= **249**
Information source	**Pubmed** (performed by ANSES)
Date accessed	22 April 2015
Search terms for STEP1 (broad search)	TITLE-ABS (β-N-Methylamino-l-alanine* OR BMAA*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	177
Search terms for STEP2 (focused search)	TITLE-ABS (β-N-Methylamino-l-alanine* OR BMAA*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2	**197**
Information source	**Scopus + Pubmed**

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Number of records retrieved	**446**
Duplicates	**191**
Number of records retrieved	**255**
Information source	**Web of science** (performed by ISS)
Date accessed	28 April 2015
Step 1 (broad search): Search terms	TOPIC (β-N-Methylamino-l-alanine* OR BMAA*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	204
Step 2 (focused search): Search terms	TOPIC (β-N-Methylamino-l-alanine* OR BMAA*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2	**242**
Information source	**Food Source Science** (performed by ISS)
Date accessed	28 April 2015
Step 1 (broad search): Search terms	TITLE-ABS-KEY (β-N-Methylamino-l-alanine* OR BMAA*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	23
Step 2 (focused search): Search terms	TITLE-ABS-KEY (β-N-Methylamino-l-alanine* OR BMAA*) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2	**29**
Information source	**Web of science (ISS) + Food Source Science (ISS)**
Number of records retrieved STEP 2	**271**
Duplicates	**26**
Number of records retrieved (duplicates removed)	**244**
Duplicates with Scopus+ Pubmed	**220**
Number of records retrieved for primary screening	**24**

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EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Ateliana SIALEHAAMOA
STUDY QUESTION	Objective 1: Search 1: Occurrence **Groups: LPS toxins**
Information source	**Scopus** (performed by ANSES)
Date accessed	22 April 2015
Search terms for STEP1 (broad search)	TITLE-ABS-KEY **(**(LPS***** OR lipopolysaccharide*****) AND (cyanobacter* OR cyanotoxin* OR blue-greenalga* OR bluealga*)) AND (presence* OR occur* OR *concentration* OR *accumulation* OR bloom* OR level* OR field* OR laboratory*))
Number of records retrieved STEP1	41
Search terms for STEP2 (focused search)	TITLE-ABS-KEY **(**(LPS***** OR lipopolysaccharide*****) AND (cyanobacter* OR cyanotoxin* OR blue-greenalga* OR bluealga*)) AND (presence* OR occur* OR *concentration* OR *accumulation* OR bloom* OR level* OR field* OR laboratory*OR food* OR "food* supplement*" OR fish* OR shellfish* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR milk* OR "dairy product*" OR vegetable* OR fruit* OR plant* OR "trophic* transfer*" OR feeding*))
Number of records retrieved STEP2	**49**
Information source	**Pubmed** (performed by ANSES)
Date accessed	22 April 2015
Search terms for STEP1 (broad search)	TITLE-ABS ((LPS* OR lipopolysaccharide*) AND (cyanobacter* OR cyanotoxin* OR blue-green alga* OR blue alga*)) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	48
Search terms for STEP2 (focused search)	TITLE-ABS ((LPS* OR lipopolysaccharide*) AND (cyanobacter* OR cyanotoxin* OR blue-green alga* OR blue alga*)) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2	**51**

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Information source	**SCOPUS + PUBMED**
Number of records retrieved	**100**
Duplicates	**24**
Number of records retrieved	**76**
Information source	**Web of science** (performed by ISS for SAXITOXINS, PALYTOXINS, CIGUATOXINS, LPS)
Date accessed	28 April 2015
Step 1 (broad search): Search terms	TOPIC ((saxitoxin* OR palytoxin* OR ciguatoxin* OR LPS* OR lipopolysaccharide*) AND (cyanobacter* OR cyanotoxin* OR blue-green alga* OR blue alga*)) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	361
Step 2 (focused search): Search terms	TOPIC ((saxitoxin* OR palytoxin* OR ciguatoxin* OR LPS* OR lipopolysaccharide*) AND (cyanobacter* OR cyanotoxin* OR blue-green alga* OR blue alga*)) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2	417
Information source	**Food Source Science** (performed by ISS)
Date accessed	28 April 2015
Step 1 (broad search): Search terms	TITLE-ABS-KEY ((saxitoxin* OR palytoxin* OR ciguatoxin* OR LPS* OR lipopolysaccharide*) AND (cyanobacter* OR cyanotoxin* OR blue-green alga* OR blue alga*)) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory*)
Number of records retrieved STEP1	33
Step 2 (focused search): Search terms	TITLE-ABS-KEY ((saxitoxin* OR palytoxin* OR ciguatoxin* OR LPS* OR lipopolysaccharide*) AND (cyanobacter* OR cyanotoxin* OR blue-green alga* OR blue alga*)) AND (presence* OR occur* OR concentration* OR bioconcentration* OR accumulation* OR bioaccumulation* OR bloom* OR level* OR field* OR laboratory* OR food* OR food supplement* OR fish* OR shellfish* OR milk* OR dairy product* OR crustacean* OR mussel* OR mollusk* OR aquatic* OR trout* OR prawn* OR seafood* OR seabird* OR eider* OR duck* OR meat* OR vegetable* OR fruit* OR plant* OR trophic transfer* OR feeding*)
Number of records retrieved STEP2	38

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Information source	**Web of science** (ISS)
Date accessed	28 April 2015
Number of records retrieved STEP1	24
Number of records retrieved STEP2	**78**
Information source	**Food Source Science** (ISS)
Date accessed	28 April 2015
Number of records retrieved STEP1	8
Number of records retrieved STEP2	**8**
Information source	**Web of science (ISS) + Food Source Science (ISS)**
Number of records retrieved STEP 2	**86**
Duplicates	**8**
Number of records retrieved (duplicates removed)	**78**
Duplicates with Scopus+ Pubmed	**56**
Number of records retrieved for primary screening	**22**

Information source form for cyanotoxins other than microcystins: compiled databases for the secondary screening

EFSA SYSTEMATIC REVIEW		Cyanobacteria toxins in food
EFSA REFERENCE		OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST		Ateliana SIALEHAAMOA and Valerie FESSARD
STUDY QUESTION		Objective 1: cyanobacteria toxins occurrence in food Search 1: Occurrence Part 2. Other cyanotoxins than microcystins
Number of records retrieved from all databases		560 (Anses) + 81 (ISS) (duplicates included) = **641**
Number of records after primary screening and after excluding duplicates		**615**
	Number of papers not included in the inventory (only abstract)	318
	Excluded (not meeting the criteria)	119
Secondary screening		
Number of records reviewed for possible inclusion in the inventory		178
	food occurrence	**92** (including publications dealing with laboratory studies for testing bioaccumulation)
	non-edible matrices	**10**
	dead animals	**11**
	reviews	**7**

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	articles on water samples, blooms and cyanobacterial cultures	**14**
	Total included in the inventory	**134**

A.3 EXISTING INTERNATIONAL/NATIONAL REGULATIONS/GUIDELINES OR "HEALTH ALERT" LEVELS

Table 3: Information source form for literature search

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Nathalie ARNICH
STUDY QUESTION	**Objective 1:** **Search 3: Monitoring systems/programs according to existing international/national regulations/guidelines or "health alert" levels**
Information source	**Scopus** (performed by ANSES)
Search terms	(TITLE-ABS-KEY (microcystin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (cyanotoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (cyanobacter* AND toxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (nodularin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (cylindrospermopsin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (anatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (homoanatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health

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	OR alert level*)); (TITLE-ABS-KEY (BMAA) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (palytoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (ciguatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (LPS toxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (*aplysiatoxin*) AND(regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (lyngbyatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*))
Date accessed (last)	9 March 2015
Number of records retrieved	661 = 221 (MCs) 82 (cyanotoxin*) 200 (cynobacter* toxin*) 23 (nodularin*) 37 (CYN) 32 (ANA) 2 (homoANA) 7 (BMAA) 13 (PlTX) 27 (CTX) 14 (LPS toxin*) 2 (*aplysiatoxin*) 1 (lyngbyatoxin*)
Duplicates	339
Duplicates excluded	**322**
Primary screening (excluded records on regulation of toxin production by cyanobacteria, recreational/bathing water monitoring, water treatment) *Many records retrieved mention "WHO guideline" for drinking water in the abstract*	**39**
Information source	**PubMed**(performed by ANSES)
Search terms	(TITLE-ABS (microcystin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*))[16]; (TITLE-ABS-KEY (cyanotoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*));

[16] (((((((microcystin*[Title/Abstract]) AND regulation*[Title/Abstract])) OR ((microcystins*[Title/Abstract]) AND management*[Title/Abstract])) OR (((microcystins*[Title/Abstract]) AND monitoring*[Title/Abstract]))) OR (((microcystins*[Title/Abstract]) AND guideline*[Title/Abstract]))) OR ((((microcystins*[Title/Abstract]) AND human health*[Title/Abstract])))) OR ((((microcystins*[Title/Abstract]) AND alert level*[Title/Abstract])))

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	(TITLE-ABS-KEY (cyanobacter* AND toxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (nodularin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (cylindrospermopsin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (anatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (homoanatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (BMAA) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (palytoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (ciguatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (LPS toxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (*aplysiatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)); (TITLE-ABS-KEY (lyngbyatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*))
Date accessed	9 March 2015
Number of records retrieved	1394 = 422 (MC) 135 (cyanotoxin*) 389 (cyanobacter* AND toxin*) 64 (nodularin*) 97 (CYN) 59 (ANA) 7 (homoANA) 20 (BMAA) 38 (PlTX) 38 (CTX) 111 (LPS toxin*) 6 (*aplysiatoxin*) 8 (lyngbyatoxin*)
Duplicates	495
Duplicates excluded	899
Primary screening (excluded records on regulation of toxin production by cyanobacteria, recreational/bathing water	58

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monitoring, water treatment) *Many records retrieved mention "WHO guideline" for drinking water in the abstract*	
Information source	**Web of Science** (performed by ISS)
Date accessed	29 April 2015
Search terms	(microcystin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) Field: TOPIC (cyanotoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) Field: TOPIC (cyanobacter* AND toxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) Field: TOPIC (nodularin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) Field: TOPIC (cylindrospermopsin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) Field: TOPIC (anatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) Field: TOPIC (homoanatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) Field: TOPIC (BMAA) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) Field: TOPIC (palytoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) Field: TOPIC (ciguatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) Field: TOPIC (LPS toxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) Field: TOPIC (*aplysiatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) Field: TOPIC (lyngbyatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) Field: TOPIC
Number of records retrieved	**4168** = 1386 (MC) 420 (cyanotoxin) 1178 (cyanobacteria) 176 (nodularin) 205 (CYN) 164 (ANA) 18 (homoANA) 56 (BMAA) 96

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	(PlTX) 135 (CTX) 297 (LPS toxin*) 17 (aplysiatoxin) 20 (lyngbyatoxin)
Information source	**Food Science Source** (performed by ISS)
Date accessed	29 April 2015
Search terms	(microcystin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) (cyanotoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) (cyanobacter* AND toxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) (nodularin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) (cylindrospermopsin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) (anatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) (homoanatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) (BMAA) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) (palytoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) (ciguatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) (*aplysiatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*) (lyngbyatoxin*) AND (regulation* OR monitoring* OR management* OR guideline* OR human health OR alert level*)
Number of records retrieved	**287**= 98 (MC) 50 (cyanotoxin) 77 (cyanobacteria) 5 (nodularin) 18 (CYN) 14 (ANA) 3 (homoANA) 3 (BMAA) 11 (PlTX) 7 (CTX)1 (lyngbyatoxin) 0 (aplysiatoxin)
Number of records retrieved from all databases, duplicates removed	92 *Many records retrieved just because they mention "WHO guideline" for drinking water in the abstract*
Number of records for secondary screening (*the full paper of all the records*	**92**

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retrieved were reviewed for possible inclusion in the inventory)	
Number of records included in the inventory	**4**
Number of records excluded because of language	5
Missing papers	9
Number of papers excluded (no useful information)	74

Table 4: Information source form for reports by agencies

EFSA SYSTEMATIC REVEAW	Review of literature on cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Nathalie ARNICH
STUDY QUESTION	**Objective 1:** **Search 3: Monitoring systems/programs according to existing international/national regulations/guidelines or "health alert" levels**
Information source	ATSDR website Toxicological profile, CDC website(performed by ANSES)
Search terms	TITLE: Microcystin Cyanotoxin Cyanobacteria Blue-green algae Nodularin Cylindrospermopsin Anatoxin Homoanatoxin BMAA Palytoxin Ciguatoxin Saxitoxin LPS toxin Aplysiatoxin Lyngbyatoxin
Date accessed	23 March 2015
Number of records retrieved	8 = 0 (MC) 0 (cyanotoxin) 3 (cyanobacteria) 5 (blue-green algae) 0 (nodularin) 0 (CYN) 0 (ANA) 0 (homoANA) 0 (BMAA) 0 (PlTX) 0 (CTX) 1 (STX) 0 (LPS toxin*) 0 (aplysiatoxin) 0 (lyngbyatoxin)
Primary screening	0
Information source	US-EPA(performed by ANSES)
Search terms	ANYWHERE IN THE DOCUMENT

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	Microcystin[17] Cyanotoxins Nodularin Cylindrospermopsin Anatoxin Homoanatoxin BMAA Palytoxin Ciguatoxin Saxitoxin LPS toxin (lipopolysaccharides) Aplysiatoxins Lyngbyatoxin
Date accessed	23, 24, 25, 26 March 2015
Number of records (links) retrieved	418 (MC) 250 (cyanotoxins) 52 (nodularin) 114 (CYN) 128 (ANA) 3 (homoANA) 10 (BMAA) 5 (PlTX) 16 (CTX) 113 (STX) 39 (LPS) 3 (Aplysiatoxisn) 10 (Lyngbyatoxin)
Primary screening	1161
Secondary screening (included in the review, but not only for regulation and monitoring)	4
Information source	WHO(performed by ANSES)
Search terms	ANYWHERE Microcystin Cyanotoxin Nodularin Cylindrospermopsin Anatoxin Homoanatoxin BMAA Palytoxin Ciguatoxin Saxitoxin LPS toxin (Lipopolysaccharides) Aplysiatoxin Lyngbyatoxin
Date accessed	26 March 2015
Number of records (links) retrieved	38 (MC) 25 (cyanotoxins) 5 (nodularin) 9 (CYN) 8 (ANA) 3 (homoANA) 0 (BMAA) 0 (PlTX) 0 (CTX) 5 (STX) 17 (LPS) 1 (Aplysiatoxisn) 2 (Lyngbyatoxin)
primary screening	113
secondary screening (included in the review, in reference to drinking water)	4
Information source	Health Canada (website)(performed by ANSES)

[17] http://nlquery.epa.gov/epasearch/epasearch?querytext=Microcystin&fld=&areaname=&areacontacts=&areasearchurl=&typeofsearch=epa&result_template=2col.ftl&force=no&filter=sample4filt.hts

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Search terms	ANYWHERE Microcystin[18] Cyanotoxin Nodularin Cylindrospermopsin Anatoxin Homoanatoxin BMAA Palytoxin Ciguatoxin Saxitoxin LPS toxin (Lipopolysaccharides) Aplysiatoxin Lyngbyatoxin
Date accessed	27 March 2015
Number of records retrieved	21 (MC) 25 (cyanotoxins) 5 (nodularin) 9 (CYN) 8 (ANA) 3 (homoANA) 0 (BMAA) 0 (PlTX) 0 (CTX) 5 (STX) 17 (LPS) 1 (Aplysiatoxisn) 2 (Lyngbyatoxin)
Primaryscreening	91
Secondary screening (included in the review, in reference to NHP and drinking water)	4
Information source	RIVM (website)(performed by ANSES)
Search terms	ANYWHERE Microcystin[19] Cyanotoxin Nodularin Cylindrospermopsin Anatoxin Homoanatoxin BMAA Palytoxin Ciguatoxin Saxitoxin LPS toxin Aplysiatoxin Lyngbyatoxin
Date accessed	7 April 2015
Number of records retrieved	2 (MC) 3 (cyanotoxin) 0 (nodularin) 0 (CYN) 2 (ANA) 0 (homoANA) 0 (BMAA) 0 (PlTX) 1 (CTX) 6 (STX) 14 (LPS) 0 (Aplysiatoxisn) 0 (Lyngbyatoxin)
Primary screening	26
Secondary screening (included in the review)	0

[18] http://recherche-search.gc.ca/rGs/s_r?st=s&s5bm3ts21rch=x&num=10&st1rt=0&langs=eng&cdn=health&q=microcystin
[19] http://www.rivm.nl/en/Search?query=microcystin

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Information source	BfR (website)(performed by ANSES)
Search terms	KEYWORDS (all topics, all words) Microcystin[20] Cyanotoxin Nodularin Cylindrospermopsin Anatoxin Homoanatoxin BMAA Palytoxin Ciguatoxin Saxitoxin LPS toxin Aplysiatoxin Lyngbyatoxin
Date accessed	7 April 2015
Number of records retrieved	0 (MC) 0 (cyanotoxin) 0 (nodularin) 0 (CYN) 0 (ANA) 0 (homoANA) 0 (BMAA) 0 (PlTX) 1 (CTX) 0 (STX) 0 (LPS) 0 (Aplysiatoxisn) 0 (Lyngbyatoxin)
Primary screening	1
Secondary screening (included in the review)	0
Information source	ISS (website)
Search terms	information provided by ISS team
Date accessed	22 May 2015
Number of records	4
Primary screening	4
Secondary screening (included in the review	0
Information source	ANSES (website)
Search terms	KEYWORDS (all topics, all words) Microcystins[21] Cyanotoxines Nodularine Cylindrospermopsine Anatoxine Homoanatoxine BMAA Palytoxine Ciguatoxines Saxitoxines LPS toxines Aplysiatoxine Lyngbyatoxine

[20] http://www.bfr.bund.de/en/search.html
[21] https://www.anses.fr/fr/search/site/microcystines?searchphrase=1&mm=100&iso1=fr&iso2=en

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	Cyanobactéries
Date accessed	7 April 2015
Number of records retrieved	2 (MC) 8 (cyanotoxines) 0 (nodularin) 0 (CYN) 0 (ANA) 0 (homoANA) 1 (BMAA) 1 (PITX) 2 (CTX) 3 (STX) 0 (LPS) 0 (Aplysiatoxisn) 0 (Lyngbyatoxin) 12 (cyanobactéries)
Primary screening	7
Secondary screening (included in the review)	3
Information source	IARC(performed by ANSES)
Search terms	(cyanobacteria OR cyanotoxin)
Date accessed	24 March 2015
Number of records retrieved	6
Secondary screening (included in the review)	1

Information sources	ANSES, ATSDR, BfR, Health Canada, IARC, ISS, US-EPA, WHO
Number of records retrieved from all sources	1409
Number of records for primary screening	1409
Number of records for secondary screening	16
Number of records included in the inventory or in the narrative description, but not only for regulation/guidelines/health alert levels	**16**

A.4 ANALYTICAL METHODS

Table 5: Information source form on analytical methods for cyanotoxins occurrebce in food

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Ronel BIRE
STUDY QUESTION	Objective 1: Cyanobacteria toxins occurrence in food Search 2: Analytical methods
Information source	**Scopus** (performed by ANSES)

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Step 1 (broad search): Search terms	TITLE-ABS-KEY (({sample preparation} OR extraction OR detection OR {analytical method}) AND (cyanobacteria OR cyanotoxin* OR microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR BMAA OR saxitoxin* OR lipopolysaccharide* OR LPS OR *aplysiatoxin* OR lyngbyatoxin* OR palytoxin* OR ovatoxin* OR ciguatoxin*))
Date accessed	17 April 2015
Number of records retrieved step 1	7735
Step 2 (focused search): Search terms	TITLE-ABS-KEY ((food OR {food supplement} OR fish OR shellfish OR crustacean* OR meat OR vegetables OR crop OR fruit* OR milk OR {dairy products}) AND ({sample preparation} OR extraction OR detection OR {analytical method}) AND (cyanobacteria OR cyanotoxin* OR microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR BMAA OR saxitoxin* OR lipopolysaccharide* OR LPS OR *aplysiatoxin* OR lyngbyatoxin* OR palytoxin* OR ovatoxin* OR ciguatoxin*))
Number of records retrieved step 2	1034
Number of records retrieved step 1 + step 2	8769
Duplicates	1089
Number of records retrieved	7680
Information source	**PubMed** (performed by ANSES)
Step 1: Search terms	TITLE-ABS(({sample preparation} OR extraction OR detection OR {analytical method}) AND (cyanobacteria OR cyanotoxin* OR microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR BMAA OR saxitoxin* OR lipopolysaccharide* OR LPS OR *aplysiatoxin* OR lyngbyatoxin* OR palytoxin* OR ovatoxin* OR ciguatoxin*))
Date accessed	17 April 2015
Number of records retrieved step 1	4013
Step 2: Search terms	TITLE-ABS((food OR {food supplement} OR fish OR shellfish OR crustacean* OR meat OR vegetables OR crop OR fruit* OR milk OR {dairy products}) AND ({sample preparation} OR extraction OR detection OR {analytical method}) AND (cyanobacteria OR cyanotoxin* OR microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR BMAA OR saxitoxin* OR lipopolysaccharide* OR LPS OR *aplysiatoxin* OR lyngbyatoxin* OR palytoxin* OR ovatoxin* OR ciguatoxin*))

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Number of records retrieved step 2	433
Number of records retrieved step 1 + step 2	4446
Duplicates	436
Number of records retrieved	4010
	Scopus + PubMed
Number of records retrieved	11690
Duplicates	3663
Number of records for 1st screening	**8027**
Information source	**Web of Science** (performed by ISS)
Step 1 (broad search): Search terms	TOPIC (("sample preparation" OR extraction OR detection OR "analytical method") AND (cyanobacteria OR cyanotoxin* OR microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR BMAA OR saxitoxin* OR lipopolysaccharide* OR LPS OR *aplysiatoxin* OR lyngbyatoxin* OR palytoxin* OR ovatoxin* OR ciguatoxin*))
Date accessed	22 April 2015
Number of records retrieved step 1	6717
Step 2 (focused search): Search terms	TOPIC ((food OR "food supplement" OR fish OR shellfish OR crustacean* OR meat OR vegetables OR crop OR fruit* OR milk OR "dairy products") AND ("sample preparation" OR extraction OR detection OR "analytical method") AND (cyanobacteria OR cyanotoxin* OR microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR BMAA OR saxitoxin* OR lipopolysaccharide* OR LPS OR *aplysiatoxin* OR lyngbyatoxin* OR palytoxin* OR ovatoxin* OR ciguatoxin*))
Number of records retrieved step 2	1032
Number of records retrieved step 1 + step 2	7749
Information source	Food science source (performed by ISS)
Step 1: Search terms	(TI ((cyanobacteria OR cyanotoxin* OR microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR BMAA OR saxitoxin* OR lipopolysaccharide* OR LPS OR *aplysiatoxin* OR lyngbyatoxin* OR palytoxin* OR ovatoxin* OR ciguatoxin*)) OR AB ((cyanobacteria OR cyanotoxin* OR microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR BMAA OR saxitoxin* OR lipopolysaccharide* OR LPS OR *aplysiatoxin*

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	OR lyngbyatoxin* OR palytoxin* OR ovatoxin* OR ciguatoxin*)) OR KW ((cyanobacteria OR cyanotoxin* OR microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR BMAA OR saxitoxin* OR lipopolysaccharide* OR LPS OR *aplysiatoxin* OR lyngbyatoxin* OR palytoxin* OR ovatoxin* OR ciguatoxin*))) AND (TI (("sample preparation" OR extraction OR detection OR "analytical method")) OR AB (("sample preparation" OR extraction OR detection OR "analytical method")) OR KW (("sample preparation" OR extraction OR detection OR "analytical method")))
Date accessed	28 April 2015
Number of records retrieved step 1	463
Step 2: Search terms	(TI ((food OR "food supplement" OR fish OR shellfish OR crustacean* OR meat OR vegetables OR crop OR fruit* OR milk OR "dairy products") OR AB ((food OR "food supplement" OR fish OR shellfish OR crustacean* OR meat OR vegetables OR crop OR fruit* OR milk OR "dairy products") OR KW ((food OR "food supplement" OR fish OR shellfish OR crustacean* OR meat OR vegetables OR crop OR fruit* OR milk OR "dairy products")) AND (TI ("sample preparation" OR extraction OR detection OR "analytical method") OR AB ("sample preparation" OR extraction OR detection OR "analytical method") OR KW ("sample preparation" OR extraction OR detection OR "analytical method")) AND (TI (cyanobacteria OR cyanotoxin* OR microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR BMAA OR saxitoxin* OR lipopolysaccharide* OR LPS OR *aplysiatoxin* OR lyngbyatoxin* OR palytoxin* OR ovatoxin* OR ciguatoxin*) OR AB (cyanobacteria OR cyanotoxin* OR microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR BMAA OR saxitoxin* OR lipopolysaccharide* OR LPS OR *aplysiatoxin* OR lyngbyatoxin* OR palytoxin* OR ovatoxin* OR ciguatoxin*) OR KW (cyanobacteria OR cyanotoxin* OR microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR BMAA OR saxitoxin* OR lipopolysaccharide* OR LPS OR *aplysiatoxin* OR lyngbyatoxin* OR palytoxin* OR ovatoxin* OR ciguatoxin*))

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Number of records retrieved step 2	101
Number of records retrieved step 1 + step 2	564
	Web of Science + Food science source
Number of records retrieved	8313
Duplicates	1346
Number of records	**6967**
Number of records retrieved in the 4 databases	14994
Duplicates	3210
Primary screening	**11874 (many duplicates not detected by Endnote)**
- Exclusion criteria: articles in Korean - Inclusion criteria: occurrence, food, food supplement, bioaccumulation, analytical method	6 **1175 (many duplicates not detected by Endnote)** **900 (after manual removal of duplicates)**
Secondary screening	
Records to be included in the inventory	127
Records reviewed not included in the inventory	349
Records not reviewed (low priority score)	424

When the name of single toxins were used, the number of the secondary screening were indicated below .

Secondary screening	
- Records to be included in the inventory	111
AnTX	14
BMAA	10
CYN	3
MC	43
NOD	14
LPS	9
CTX	18
PLTX	15
STX	15
Multi-toxin	4
- Records reviewed not included in the inventory	
AnTX	26
BMAA	34
CYN	14

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MC	82
NOD	30
LPS	5
CTX	2
PLTX	3
STX	11
Multi-toxin	29
Reviews	20
Papers not found	12

A.5 TOXICOLOGICAL PROFILE (Hazard identification and Characterization)

Table 6: Information source form for **Microcystins** Search 1 and 2 (Toxicity, Toxicokinetics, epidemiology)

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Franca M. Buratti/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 1and 2: Toxicity, Toxicokinetics, epidemiology** **Microcystins**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY(epidemiology OR toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (microcystin* OR "MC-LR" OR "MC-YR" OR "MC-RR" OR "MC-AR" OR "MC-LF" OR "MC-LW" OR "MC-FR" OR "MC-deLR" OR "MC-deRR" OR "MC-deLW" OR "MC-deLF" OR "MC-(O)Y" OR "MC-m[Glu(OCH_3)]-LR")

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Number of records retrieved	**2696**
Information source	**PubMed** (performed by ISS)
Last Date accessed	21/05/2015
Search terms	ALL FIELDS(epidemiology OR toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (microcystin*)
Number of records retrieved	**2413**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	14/05/2015
Search terms	TOPIC,from all databases(epidemiology OR toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (microcystin* OR "MC-LR" OR "MC-YR" OR "MC-RR" OR "MC-AR" OR "MC-LF" OR "MC-LW" OR "MC-FR" OR "MC-deLR" OR "MC-deRR" OR "MC-deLW" OR "MC-deLF" OR "MC-(O)Y" OR "MC-m[Glu(OCH3)]-LR")
Number of records retrieved	**3253**
Information source	**Agricola** (performed by ISS)
Last Date accessed	15/05/2015
Search terms	TITLE-ABS-KEY (epidemiology OR toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (microcystin* OR "MC-LR" OR

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	"MC-YR" OR "MC-RR" OR "MC-AR" OR "MC-LF" OR "MC-LW" OR "MC-FR" OR "MC-deLR" OR "MC-deRR" OR "MC-deLW" OR "MC-deLF" OR "MC-(O)Y" OR "MC-m[Glu(OCH3)]-LR")
Number of records retrieved	**403**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	15/05/2015
Search terms	TITLE-ABS-KEY (epidemiology OR toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (microcystin* OR "MC-LR" OR "MC-YR" OR "MC-RR" OR "MC-AR" OR "MC-LF" OR "MC-LW" OR "MC-FR" OR "MC-deLR" OR "MC-deRR" OR "MC-deLW" OR "MC-deLF" OR "MC-(O)Y" OR "MC-m[Glu(OCH3)]-LR")
Number of records retrieved	**196**
Information source	Records coming from 1) Search 3 and 4; 2) From Search 1&2 on other toxins 3) from ANSES related to i.p. injection
Number of records retrieved	**1) 77** **2) 75** **3) 56** **Total 208**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**9169**
Duplicates	5393
Number of records for primary screening	**3776**
Deleted due to exclusion criteria (1st screening)	**Total: 2634** Language 108 Plants and invertebrates 353 Other cyanotoxins 214 Off topic (no cyanotoxins; other objectives, including occurrence) 1959
Number of records for secondary screening	**Total 1149** **(**1142 **+ 7 derived from more than 1 toxins data base /other searches)**

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Further excluded during secondary Screening	**Total: 389** K4[22]: 35 Language:9 Crude extracts/uncharacterized extracts:136 Only occurrence:44 Off topic (e.g. toxin used as probe):80 Other toxins/search/ more toxins 80 Articles not available: 5
Reviews	**122** Not eligible for data extraction
Risk assessment /Reviews	**73** Not eligible for data extraction
Number of records eligible for data extraction	**565**
Number of records extracted	**82**
Subdivision of extracted records	
Acute Toxicity	**17**
ADME	**6**
Epidemiology/human	**10**
Genotox in vitro	**9**
Genotox in vivo	**8**
More toxins/search 4	**6**
Repeated Toxicity/carcinog	**16**
Reprotox in vivo	**10**
Subdivision of the records eligible but not extracted due to low priority criteria for data extraction	
Acute toxicity (no reference dose)	22
ADME in vitro (Absorption)	10
ADME in vitro (Distribution)	2
ADME in vitro (Elimination)	0
ADME in vitro (Metabolism)	4
ADME in vitro/qualitative	2
In vitro citotoxicity	64
Toxicity study on Birds	4
Repeated toxicity (one dose tested)	7
Reprotoxicity in vitro	4
MOA organism	1
MOA organ	4
MOA (cellular target)	31
MOA Molecular target	98
MOA fish	27
Acute toxicity fish	43
ADME fish	41

[22] K4 is the abbreviation for Klimish score 4 (regarding quality of the paper)

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In vitro fish	14
Repeated toxicity in fish	42
Reprotoxicity in fish/Xenopus	21
Animal poisoning	37
Case study (human intoxication)	1
Multiple case study/outbreaks (no structured epidemiological study)	4

Table 7: Information source form for **Nodularins** Search 1 and 2

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Maura Manganelli/Franca M. Buratti
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 1and 2: Toxicity, Toxicokinetics, epidemiology** **Nodularins**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY(toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **AND** (nodularin*)
Number of records retrieved	**329**
Information source	**PubMed** (performed by ISS)
Last Date accessed	26/05/2015
Step 1: Search terms	ALL FIELDS(toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (nodularin*)
Number of records retrieved	**229**

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Information source	**Web of Science** (performed by ISS)
Last Date accessed	26/05/2015
Search terms	TOPIC: ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (nodularin*))
Number of records retrieved	**366**
Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	(TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR Descriptor ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) AND (TI (nodularin*) OR AB (nodularin*) OR Descriptor (nodularin*))
Number of records retrieved	**1**
Information source	**Food Science Source** (performed by ISS)

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Last Date accessed	26/05/2015
Search terms	(TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR KW ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) AND (TI (nodularin*) OR AB (nodularin*) OR KW (nodularin*))
Number of records retrieved	**16**
Information source	Records coming from other searches
Number of records retrieved	**34**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**975**
Duplicates	502
Number of records for primary screening	**473**
Deleted due to exclusion criteria (1st screening)	**Total: 330** Language 2 Plants and invertebrates 25

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	Off topic (no cyanotoxins; other objectives, including occurrence) 303
Number of records for secondary screening	**Total: 147** **143 +4 derived from More toxins/search 4**
Further excluded during secondary Screening	**Total: 79** K4: 5 Crude extracts/uncharacterized extracts:2 Off topic (e.g. toxin used as probe, occurrence):72
Reviews	**40** Not eligible for data extraction
Risk assessment /Reviews	**5** Not eligible for data extraction
Number of records eligible for data extraction	**23**
Number of records extracted	**12**
Subdivision of extracted records	
Genotox	**1**
In vivo	**7**
More toxins/search 4 in vivo	**4**
Subdivision of the records eligible but not extracted due to low priority criteria for data extraction	
In vivo fish	1
In vitro	1
MOA fish	1
MOA rodents (Molecular target)	6
Animal Poisoning	2

Table 8: Information source form for **Anatoxins** Search 1 and 2

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Maura Manganelli/Franca M. Buratti
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 1and 2: Toxicity, Toxicokinetics, epidemiology** **Anatoxins**
Information source	**Scopus** (performed by ANSES)

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Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY(toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (anatoxin* OR homoanatoxin*)
Number of records retrieved	**536**
Information source	**PubMed** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	ALL FIELDS (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (anatoxin* OR homoanatoxin*)
Number of records retrieved	**452**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	26/05/2015
Search terms	TOPIC: ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (anatoxin* OR homoanatoxin*))
Number of records retrieved	**576**
Information source	**Agricola** (performed by ISS)
Last Date accessed	26/05/2015
Search terms	(TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP

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	OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR Descriptor ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) AND (TI (anatoxin* OR homoanatoxin*) OR AB (anatoxin* OR homoanatoxin*) OR DE (anatoxin* OR homoanatoxin*))
Number of records retrieved	**23**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	15/05/2015
Search terms	(TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR

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	epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR Descriptor ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) AND (TI (anatoxin* OR homoanatoxin*) OR AB (anatoxin* OR homoanatoxin*) OR DE (anatoxin* OR homoanatoxin*))
Number of records retrieved	**18**
Information source	Records coming from other Searches
Number of records retrieved	**78**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**1683**
Duplicates	850
Number of records for primary screening	**833**
Deleted due to exclusion criteria (1st screening)	**Total: 664** Language 7 Plants and invertebrates 23 Off topic (no cyanotoxins; other objectives, including occurrence) 634
Number of records for secondary screening	**Total: 175** **(169 + 6 derived from More than 1 toxins/ search 4)**
Further excluded during secondary Screening	**Total: 48** K4: 2 Crude extracts/uncharacterized extracts:6 Off topic (e.g. toxin used as probe, occurrence only):33 Articles not available: 7
Reviews	**56** Not eligible for data extraction
Risk assessment /Reviews	**12** Not eligible for data extraction
Number of records eligible for data extraction	**59**
Number of extracted records	**17**
Subdivision of extracted records	

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ATX in vivo	6
ATX-(a)-S in vitro	1
ATX-(a)-S in vivo	7
More toxins/search 4 in vivo	3
Subdivision of the records eligible but not extracted due to low priority criteria for data extraction	
ATX in vivo no reference dose	3
ATX in vitro	1
MOA ATX Molecular target	17
ATX in vivo fish	2
MOA ATX fish	5
Animal poisoning	14

Table 9: Information source form for **Cylindrospermopsin** Search 1 and 2 (Toxicity, Toxicokinetics, epidemiology)

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Franca M. Buratti /Maura Manganelli
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 1and 2: Toxicity, Toxicokinetics, epidemiology** **Cylindrospermopsin**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY (TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR

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	transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR Descriptor ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) AND (TI (cylindrospermopsin*) OR AB (cylindrospermopsin*) OR DE (cylindrospermopsin*))
Number of records retrieved	**339**
Information source	**PubMed** (performed by ISS)
Last Date accessed	21/05/2015
Search terms	ALL FIELDS (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (cylindrospermopsin*)
Number of records retrieved	**284**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TOPIC: ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (cylindrospermopsin*))

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Number of records retrieved	**410**
Information source	**Agricola** (performed by ISS)
Last Date accessed	21/05/2015
Search terms	TITLE-ABS-KEY (TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR Descriptor ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) AND (TI (cylindrospermopsin*) OR AB (cylindrospermopsin*) OR DE (cylindrospermopsin*))
Number of records retrieved	**41**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY (TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME)

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	OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR Descriptor ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) AND (TI (cylindrospermopsin*) OR AB (cylindrospermopsin*) OR DE (cylindrospermopsin*))
Number of records retrieved	**25**
Information source	Records coming from other Searches
Number of records retrieved	**67**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**1166**
Duplicates	680
Number of records for primary screening	**486**
Deleted due to exclusion criteria (1st screening)	**Total: 308** Language 2 Plants and invertebrates 31 Off topic (no cyanotoxins; other objectives, including occurrence) 275
Number of records for secondary screening	**Total: 180** (178 + 2 derived from more than 1 toxins/search 4/in vivo&in vitro records)
Further excluded during secondary Screening	**Total: 65** K4: 6 Crude extracts/uncharacterized extracts:13 Off topic (e.g. toxin used as probe, occurrence only):46
Reviews	**42** Not eligible for data extraction
Risk assessment /Reviews	**9** Not eligible for data extraction

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Number of records eligible for data extraction	**64**
Number of extracted records	**20**
Subdivision of extracted records	
In vivo	11
Genotoxicity	8
More toxins /search 4	1
Subdivision of the records eligible but not extracted due to low priority criteria for data extraction	
Acute no reference dose	3
ADME in vitro	4
In vitro	6
Reprotox in vitro	3
MOA cellular/molecular target	14
In vivo fish	6
In vitro fish	1
MOA fish	4
Animal Poisoning	2
By products	1

Table 10: Information source form for **Saxitonins** Search 1 and 2 (Toxicity, Toxicokinetics, epidemiology)

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi /Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 1and 2: Toxicity, Toxicokinetics, epidemiology** **Saxitoxins**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY (TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR

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	"reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR Descriptor ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) **AND** (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Title OR** (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Abstract OR** (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Keywords** **Time constraint: starting from 1/1/2009**
Number of records retrieved	**347**
Information source	**PubMed** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	ALL FIELDS (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Time constraint: starting from 1/1/2009**
Number of records retrieved	**221**

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Information source	**Web of Science** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TOPIC: ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Time constraint: starting from 1/1/2009**
Number of records retrieved	**362**
Information source	**Agricola** (performed by ISS)
Last Date accessed	21/05/2015
Search terms	TITLE-ABS-KEY (TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR Descriptor ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term"

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	OR acute OR "long-term" OR chronic))) AND (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Title OR** (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Abstract OR** (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Descriptors** **Time constraint: starting from 1/1/2009**
Number of records retrieved	**45**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY (TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR Descriptor ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) AND (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Title OR** (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Abstract OR** (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Descriptors** **Time constraint: starting from 1/1/2009**

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Number of records retrieved	**26**
Information source	Records coming from other Searches
Number of records retrieved	**0**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**1001**
Duplicates	531
Number of records for primary screening	**469**
Deleted due to exclusion criteria (1st screening) or off topic during 2nd screening	**Total: 306** Language Plants and invertebrates Off topic (no cyanotoxins; other objectives, including occurrence)
Number of records for secondary screening	**163**
Further excluded during data extraction	**Total: 8** K4_extract not quantified/crude extracts/short communications
Reviews	**82** Not eligible for data extraction
REDIRECTED to other searches	**Total: 38** more than 1 toxin: 6 other toxins : 6 search 3: 19 search 4: 7
Number of records eligible for data extraction	**35**
Number of extracted records	**10**
Subdision of extracted records	
In vivo_	6
In vitro/ADME	2
In vitro/GENOTOX	1
Epidemiological studies	1
Subdivision of the records eligible but not extracted due to low priority criteria for data extraction	
In vitro	4
Case report	2
Epidemiological/article not available	1
MoA_IN VITRO	1
MoA_IN VIVO	6
fish	11

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Table 11: Information source form for **LPS** Search 1 and 2 (Toxicity, Toxicokinetics, epidemiology)

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 1and 2: Toxicity, Toxicokinetics, epidemiology** **LPS**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Title OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Abstract OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Keywords AND** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Title OR**

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	((lipopolysaccharide OR LPS) AND cyanobacteria) **Abstract OR** ((lipopolysaccharide OR LPS) AND cyanobacteria)**Keywords**
Number of records retrieved	**85**
Information source	**PubMed** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	**ALL FIELDS** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND ((lipopolysaccharide OR LPS) AND cyanobacteria)
Number of records retrieved	**71**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TOPIC,from all databases(toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND ((lipopolysaccharide OR LPS) AND cyanobacteria)
Number of records retrieved	**75**
Information source	**Agricola** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)

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	Title OR (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Abstract OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Keywords AND** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Title OR** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Abstract OR** ((lipopolysaccharide OR LPS) AND cyanobacteria)**Keywords**
Number of records retrieved	**3**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Title OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Abstract OR** (toxicokinetic OR

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	(absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Keywords AND** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Title OR** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Abstract OR** ((lipopolysaccharide OR LPS) AND cyanobacteria)**Keywords**
Number of records retrieved	**4**
Information source	Records coming from other Searches:
Number of records retrieved	**0**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**238**
Duplicates	98
Number of records for primary screening	**126**
Deleted due to exclusion criteria (1st screening) or off topic after 2nd screening	**67**
Number of records for secondary screening	**59**
Further excluded during secondary Screening	**Total: 4** K4
Reviews	**27** Not eligible for data extraction
Redirected to other searches	**Total: 22** more than 1 toxin : 5 other toxins: 9 search 3: 1 search 4: 7
Number of records eligible for data extraction	**6**
Number of extracted records	**5**
Subdision of extracted records	
In vivo	3
In vitro	2

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Subdivision of the records eligible but not extracted due to low priority criteria for data extraction	
case study_2nd priority	1

Table 12: Information source form for **BMAA** Search 1 and 2 (Toxicity, Toxicokinetics, epidemiology)

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 1and 2: Toxicity, Toxicokinetics, epidemiology** **BMAA**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	25/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) Title OR (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) Abstract OR (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME)

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	OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) Keywords AND(BMAA OR beta-Methylamino-L-alanine) Title OR (BMAA OR beta-Methylamino-L-alanine) Abstract OR (BMAA OR beta-Methylamino-L-alanine) Keywords
Number of records retrieved	**206**
Information source	**PubMed** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	**ALL FIELDS**(toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (BMAA OR beta-Methylamino-L-alanine)
Number of records retrieved	**257**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TOPIC,from all databases(toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (BMAA OR beta-Methylamino-L-alanine)
Number of records retrieved	**229**
Information source	**Agricola** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR

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	(distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (BMAA OR beta-Methylamino-L-alanine) Title OR (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (BMAA OR beta-Methylamino-L-alanine) Abstract OR ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (BMAA OR beta-Methylamino-L-alanine) DescriptorsAND(BMAA OR beta-Methylamino-L-alanine) Title OR (BMAA OR beta-Methylamino-L-alanine) Abstract OR (BMAA OR beta-Methylamino-L-alanine) Descriptors
Number of records retrieved	**9**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) Title OR (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR

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	"glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) Abstract OR (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) Keywords AND(BMAA OR beta-Methylamino-L-alanine) Title OR (BMAA OR beta-Methylamino-L-alanine) Abstract OR (BMAA OR beta-Methylamino-L-alanine) Keywords
Number of records retrieved	**13**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**714**
Duplicates	403
Number of records for primary screening	**311**
Deleted due to exclusion criteria (1st screening) or off topic during 2nd screening	**159**
Number of records for secondary screening	**Total: 156** (154+ 2 coming from other searches)
Further excluded during secondary Screening	**Total: 13** BMAA as a probe : 5 K4_toxin not quantified, poor data: 8
Reviews	**61** Not eligible for data extraction
Redirected to other searches	**Total: 4** more than 1 toxin :1 search 3 :1 search 4 :2
Number of records	**78**

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eligible for data extraction	
Number of extracted records	**12**
Subdision of extracted records	
ADME_in vitro	2
ADME_in vivo	8
Epidemiological	2
Subdivision of the records eligible but not extracted due to low priority criteria for data extraction	
Case study	3
Human post mortem	3
In vitro	15
In vivo (1 dose tested)	6
MoA (organ level)	9
MoA_IN VIVO/cellular	3
MoA_IN VIVO/molecular	9
MoA_EX VIVO	6
MoA_IN VITRO	10
fish	2

Table 13: Information source form for **Aplysiatoxin** Search 1 and 2 (Toxicity, toxicokinetics, epidemiology)

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 1and 2: Toxicity, Toxicokinetics, epidemiology** **Aplysiatoxin**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	25/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR

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	CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) Title OR (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) Abstract OR (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) Keywords AND (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Title OR (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Abstract OR (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Keywords
Number of records retrieved	**129**
Information source	**PubMed** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	**ALL FIELDS**(toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR

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	anhydrodebromoaplysiatoxin*)
Number of records retrieved	**110**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TOPIC, from all databases (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*)
Number of records retrieved	**121**
Information source	**Agricola** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Title OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Abstract OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive

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	toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Descriptors AND** (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) **Title OR** (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) **Abstract OR** (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) **Descriptors**
Number of records retrieved	**3**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) Title OR (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) Abstract OR (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR

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	"long-term" OR chronic) Keywords AND(aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*)**Title OR**(aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*)**Abstract OR**(aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*)**Keywords**
Number of records retrieved	**2**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**365**
Duplicates	198
Number of records for primary screening	**171**
Deleted due to exclusion criteria (1st screening) or off topic after 2nd screening	**93**
Number of records for secondary screening	**78**
Further excluded during secondary Screening	**Total: 3** K4 - short communication/scarce quality
Reviews	**23** Not eligible for data extraction
Redirected to other searches	more than 1 toxin : 9 search 4 :3
Number of records eligible for data extraction	**Total: 40**
Number of records extracted	**7**
Subdision of extracted records	
in vitro	2
in vivo	5
Subdivision of the records eligible but not extracted due to low priority criteria for data extraction	
In vitro	2
In vivo_not available articles	3
MoA_IN VITRO	22
MoA_IN VIVO/organ	3
Case study	3

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Table 14: Information source form for **Lyngbyatoxin** Search 1 and 2 (Toxicity, Toxicokinetics, epidemiology)

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 1and 2: Toxicity, Toxicokinetics, epidemiology** **Lyngbyatoxin**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	25/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Title OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Abstract OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Keywords AND** (lyngbyatoxin*) **Title OR** (lyngbyatoxin*) **Abstract OR**

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	(lyngbyatoxin*) **Keywords**
Number of records retrieved	**101**
Information source	**PubMed** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	**ALL FIELDS**(toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (lyngbyatoxin*)
Number of records retrieved	**54**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TOPIC, from all databases (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (lyngbyatoxin*)
Number of records retrieved	**61**
Information source	**Agricola** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Title OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR

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	OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Abstract OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Descriptors AND** (lyngbyatoxin*) **Title OR** (lyngbyatoxin*) **Abstract OR** (lyngbyatoxin*) **Descriptors**
Number of retrieved records	**2**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Title OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Abstract OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR

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	intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Keywords AND** (lyngbyatoxin*) **Title OR** (lyngbyatoxin*)**Abstract OR** (lyngbyatoxin*) **Keywords**
Number of records retrieved	**2**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**220**
Duplicates	95
Number of records for primary screening	**125**
Deleted due to exclusion criteria (1st screening) or off topic after 2nd screening	**66**
Number of records for secondary screening	**Total: 64** (60 + 4 coming from other searches)
Further excluded during secondary Screening	**Total: 4** K4_cyanobacterial extract - toxin not quantified
Reviews	**24** Not eligible for data extraction
Number of records eligible for data extraction	**Total: 36** (30 + 6 coming from other searches)
Redirected	more than 1 toxin: 15 other toxins: 1 search 4: 1
Number of extracted records	**6**
Subdision of extracted records	
in vitro	3
in vivo	3
Subdivision of the records eligible but not extracted due to low priority criteria for data extraction	
Case report	1
In vivo_articles not found	1
MoA_EX VIVO	1
MoA_IN VITRO	9
MoA_IN VIVO	1

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Table 15: Information source form for **Palytoxin** Search 1 and 2 (Toxicity, Toxicokinetics, epidemiology)

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 1and 2: Toxicity, Toxicokinetics, epidemiology** **Palytoxin**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Title OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Abstract OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Keywords AND** **(**palytoxin* OR PlTX OR ostreocin*) Title OR (palytoxin* OR PlTX OR ostreocin*) Abstract OR (palytoxin* OR PlTX OR ostreocin*) Keywords

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	Time constraint: starting from 1/1/2009
Number of records retrieved	**120**
Information source	**PubMed** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	**ALL FIELDS**(toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (palytoxin* OR PlTX OR ostreocin*) **Time constraint: starting from 1/1/2009**
Number of records retrieved	**111**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TOPIC, from all databases (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) AND (palytoxin* OR PlTX OR ostreocin*) **Time constraint: starting from 1/1/2009**
Number of records retrieved	**160**
Information source	**Agricola** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR

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	mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Title OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Abstract OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Descriptors AND** (palytoxin* OR PlTX OR ostreocin*) **Title OR** (palytoxin* OR PlTX OR ostreocin*) **Abstract OR** (palytoxin* OR PlTX OR ostreocin*) **Descriptors** **Time constraint: starting from 1/1/2009**
Number of records retrieved	**9**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Title OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR

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	thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Abstract OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Keywords AND** (palytoxin* OR PlTX OR ostreocin*) **Title OR** (palytoxin* OR PlTX OR ostreocin*) **Abstract OR** (palytoxin* OR PlTX OR ostreocin*) **Keywords** **Time constraint: starting from 1/1/2009**
Number of records retrieved	**9**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**409**
Duplicates	224
Number of records for primary screening	**Total: 188** (185 + 3 imported from other searches)
Deleted due to exclusion criteria (1st screening) or off topic during 2nd screening	**91**
Number of records for secondary screening/data extraction	**97**
Further excluded during secondary Screening	**Total: 5** K4_extract not quantified/crude extracts/short communications
Reviews	**37** Not eligible for data extraction
Redirected to other searches	**Total: 16** more than 1 toxin :1 search 3: 12 search 4 :3
Number of records eligible for data extraction	**39**
Number of extracted records	**8**
Subdision of extracted records	
In vitro	3
In vivo	5
Subdivision of the records eligible but not extracted due to low priority criteria for data	

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extraction	
Case study	7
Ex vivo	1
in vitro	8
MoA_IN VITRO	13
MoA_IN VIVO/molecular	1
Fish	1

Table 16: Information source form for **Ciguatoxin** Search 1 and 2 (Toxicity, Toxicokinetics, epidemiology)

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 1and 2: Toxicity, Toxicokinetics, epidemiology** **Ciguatoxin**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Title OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Abstract OR** (toxicokinetic OR

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	(absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Keywords AND** (*ciguatoxin* OR (CTX AND cyanobacteria)) **Title OR** (*ciguatoxin* OR (CTX AND cyanobacteria)) **Abstract OR** (*ciguatoxin* OR (CTX AND cyanobacteria)) **Keywords** **Time constraint: starting from 1/1/2009**
Number of records retrieved	**74**
Information source	**PubMed** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	**ALL FIELDS**(toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (*ciguatoxin* OR (CTX AND cyanobacteria)) **Time constraint: starting from 1/1/2009**
Number of records retrieved	**110**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TOPIC, from all databases (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) AND (*ciguatoxin* OR (CTX AND cyanobacteria)) **Time constraint: starting from 1/1/2009**
Number of records retrieved	**129**

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Information source	**Agricola** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Title OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Abstract OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Descriptors AND** (*ciguatoxin* OR (CTX AND cyanobacteria)) **Title OR** (*ciguatoxin* OR (CTX AND cyanobacteria)) **Abstract OR** (*ciguatoxin* OR (CTX AND cyanobacteria)) **Descriptors** **Time constraint: starting from 1/1/2009**
Number of records retrieved	**14**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	TITLE-ABS-KEYWORDS: (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR

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	epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Title OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Abstract OR** (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR epidemiology OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) **Keywords AND** (*ciguatoxin* OR (CTX AND cyanobacteria)) **Title OR** (*ciguatoxin* OR (CTX AND cyanobacteria)) **Abstract OR** (*ciguatoxin* OR (CTX AND cyanobacteria)) **Keywords** **Time constraint: starting from 1/1/2009**
Number of records retrieved	**9**
Information source	Records coming from other Searches:
Number of records retrieved	**0**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**336**
Duplicates	168
Number of records for primary screening	**168**
Deleted due to exclusion criteria (1st screening) or off topic during 2nd screening	**105**
Number of records for secondary screening/data extraction	**63**
Further excluded during	**Total: 8**

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secondary Screening/data extraction	crude extract/toxin not quantified - K4
Reviews	**21** Not eligible for data extraction
Redirected	**Total: 6** more than 1 toxin: 2 search 3 :2 search 4: 2
Number of records eligible for data extraction	**Total=28**
Number of extracted records	**5**
Subdision of extracted records	
In vivo	2
Epidemiological	3
Subdivision of the records eligible but not extracted due to low priority criteria for data extraction	
Case report	8
Dinoflagellate	2
In vitro	4
MoA_IN VITRO	9

Table 17: Information source form for **Cyanobacterial extracts** Search 1 and 2 (Toxicity, Toxicokinetics, epidemiology)

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Maura Manganelli/Franca M. Buratti
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 1and 2: Toxicity, Toxicokinetics, epidemiology** **Cyanobacterial extracts**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: (TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR

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	"reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR KW ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) AND (TI (cyanobacteria* extract) OR AB (cyanobacteria* extract) OR KW (cyanobacteria* extract))
Number of records retrieved	**108**
Information source	**PubMed** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	**ALL FIELDS**ALL FIELDS (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND ("cyanobacteria extract")[23]
Number of records retrieved	**33**

[23] In the case of PubMed and WebOfScience the search was performed using "Cyanobacteria extract", because the asterisk at the end of the word was misleading. This is true for all the searches, also 3 and 4.

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Information source	**Web of Science** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	**TOPIC:** ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND ("cyanobacteria extract"))
Number of records retrieved	**113**
Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: (TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR KW ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) AND (TI (cyanobacteria* extract) OR AB (cyanobacteria* extract) OR DE (cyanobacteria*

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	extract))
Number of records retrieved	**19**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: (TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR KW ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) AND (TI (cyanobacteria* extract) OR AB (cyanobacteria* extract) OR KW (cyanobacteria* extract))
Number of records retrieved	**5**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**278**
Duplicates	121
Number of records for primary screening	**157**
Deleted due to exclusion criteria (1st screening)	**92**

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Number of records for secondary screening	**65**
	Since single toxins were determined in all the papers eligible for secondary screening, records were allocated to the specific toxin group and immediately removed being a duplicate. Therefore this search gave not results for the secondary screening

Table 18: Information source form for **Cyanotoxins** 'NOT'[24] Search 1 and 2 (Toxicity, Toxicokinetics, epidemiology)

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Maura Manganelli/Franca M. Buratti
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 1and 2: Toxicity, Toxicokinetics, epidemiology** **Cyanotoxins NOT**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEYWORDS: (TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR

[24] The search includes the term Cyanotoxin(s) connected by the boolean connector NOT with the name of single toxins (that is the object of other searches, as described previously)

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	thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR KW ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) AND (TI (cyanotoxin* AND NOT (microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR bmaa OR beta-n-methylamino-l-alanine OR *saxitoxin* OR lyngbyatoxin* OR lipopolysaccharide* OR lps OR *aplysiatoxin* OR palytoxin* OR pltx OR ostreocin* OR *ciguatoxin*) OR AB (cyanotoxin* AND NOT (microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR bmaa OR beta-n-methylamino-l-alanine OR *saxitoxin* OR lyngbyatoxin* OR lipopolysaccharide* OR lps OR *aplysiatoxin* OR palytoxin* OR pltx OR ostreocin* OR *ciguatoxin*) OR KW (cyanotoxin* AND NOT (microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR bmaa OR beta-n-methylamino-l-alanine OR *saxitoxin* OR lyngbyatoxin* OR lipopolysaccharide* OR lps OR *aplysiatoxin* OR palytoxin* OR pltx OR ostreocin* OR *ciguatoxin*))
Number of records retrieved	**58**
Information source	**PubMed** (performed by ISS)
Last Date accessed	22/05/2015
Search terms	**ALL FIELDS**ALL FIELDS (toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (cyanotoxin* AND NOT (microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR bmaa OR beta-n-methylamino-l-alanine OR *saxitoxin* OR lyngbyatoxin* OR lipopolysaccharide* OR lps OR *aplysiatoxin* OR palytoxin* OR pltx OR ostreocin* OR *ciguatoxin*))
Number of records retrieved	**38**
Information source	**Web of Science** (performed by ISS)

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Last Date accessed	27/05/2015
Search terms	**TOPIC:** ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic) AND (cyanotoxin* AND NOT (microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR bmaa OR beta-n-methylamino-l-alanine OR *saxitoxin* OR lyngbyatoxin* OR lipopolysaccharide* OR lps OR *aplysiatoxin* OR palytoxin* OR pltx OR ostreocin* OR *ciguatoxin*))
Number of records retrieved	**62**
Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: (TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR KW ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR

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	thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) AND (cyanotoxin* AND NOT (microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR bmaa OR beta-n-methylamino-l-alanine OR *saxitoxin* OR lyngbyatoxin* OR lipopolysaccharide* OR lps OR *aplysiatoxin* OR palytoxin* OR pltx OR ostreocin* OR *ciguatoxin*))OR AB (cyanotoxin* AND NOT (microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR bmaa OR beta-n-methylamino-l-alanine OR *saxitoxin* OR lyngbyatoxin* OR lipopolysaccharide* OR lps OR *aplysiatoxin* OR palytoxin* OR pltx OR ostreocin* OR *ciguatoxin*))OR KW(cyanotoxin* AND NOT (microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR bmaa OR beta-n-methylamino-l-alanine OR *saxitoxin* OR lyngbyatoxin* OR lipopolysaccharide* OR lps OR *aplysiatoxin* OR palytoxin* OR pltx OR ostreocin* OR *ciguatoxin*)))
Number of records retrieved	**22**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: (TI ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR AB ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic)) OR KW ((toxicokinetic OR (absorption and ADME) OR (distribution and ADME) OR metabolism OR (excretion and ADME) OR ADME OR OATP OR transporters OR intestinal OR BBB OR "blood brain barrier" OR "placental barrier" OR "glutathione transferases" OR CYP OR "cytochrome P450" OR cytotoxicity OR toxicity OR "adverse effects" OR mortality OR genotoxicity OR carcinogenicity OR "reproductive toxicity" OR neurotoxicity OR immunotoxicity OR vitro OR "in vivo" OR epidemiology OR liver OR kidney OR sperm OR ovary OR testicles OR

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	thyroid OR brain OR "mode of action" OR mechanism OR "short-term" OR acute OR "long-term" OR chronic))) AND (TI (cyanotoxin* AND NOT (microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR bmaa OR beta-n-methylamino-l-alanine OR *saxitoxin* OR lyngbyatoxin* OR lipopolysaccharide* OR lps OR *aplysiatoxin* OR palytoxin* OR pltx OR ostreocin* OR *ciguatoxin*))OR AB (cyanotoxin* AND NOT (microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR bmaa OR beta-n-methylamino-l-alanine OR *saxitoxin* OR lyngbyatoxin* OR lipopolysaccharide* OR lps OR *aplysiatoxin* OR palytoxin* OR pltx OR ostreocin* OR *ciguatoxin*))OR KW (cyanotoxin* AND NOT (microcystin* OR nodularin* OR cylindrospermopsin* OR *anatoxin* OR bmaa OR beta-n-methylamino-l-alanine OR *saxitoxin* OR lyngbyatoxin* OR lipopolysaccharide* OR lps OR *aplysiatoxin* OR palytoxin* OR pltx OR ostreocin* OR *ciguatoxin*)))
Number of records retrieved	**24**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**204**
Duplicates	84
Number of records for primary screening	**120**
Deleted due to exclusion criteria (1st screening)	**87** Plants and invertebrate: 14 Off topic: 73
Number of records for secondary screening	**33**
	Since all the records eligible for secondary screening correspond to review papers, not eligible for data extraction, this search was further considered.

A.6 ENVIRONMENTAL FACTORS AFFECTING CYANOBACTERIAL TOXICITY

Table 19: Information source form for **Microcystins** Search 3

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Franca M. Buratti/Maura Manganelli

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STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 3: Environmental factors affecting cyanotoxin toxicity** **Microcystins**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (microcystin* OR “MC-LR” OR “MC-YR” OR “MC-RR” OR “MC-AR” OR “MC-LF” OR “MC-LW” OR “MC-FR” OR “MC-deLR” OR “MC-deRR” OR “MC-deLW” OR “MC-deLF” OR “MC-(O)Y” OR “MC-m[Glu(OCH3)]-LR”)
Number of records retrieved	**215**
Information source	**PubMed** (performed by ISS)
Last Date accessed	21/05/2015
Search terms	ALL FIELDS((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (microcystin*)
Number of records retrieved	**185**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	14/05/2015
Search terms	TOPIC, from all data bases((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (microcystin* OR “MC-LR” OR “MC-YR” OR “MC-RR” OR “MC-AR” OR “MC-LF” OR “MC-LW” OR “MC-FR” OR “MC-deLR” OR “MC-deRR” OR “MC-deLW” OR “MC-deLF” OR “MC-(O)Y” OR “MC-m[Glu(OCH3)]-LR”)
Number of records retrieved	**261**
Information source	**Agricola** (performed by ISS)
Last Date accessed	15/05/2015
Search terms	TITLE-ABS-KEY ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (microcystin* OR “MC-LR” OR “MC-YR” OR “MC-RR” OR “MC-AR” OR “MC-LF” OR “MC-LW” OR “MC-FR” OR “MC-deLR” OR “MC-deRR” OR “MC-deLW” OR “MC-deLF” OR “MC-(O)Y” OR “MC-m[Glu(OCH3)]-LR”)
Number of records retrieved	**46**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	15/05/2015
Search terms	TITLE-ABS-KEY ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (microcystin* OR “MC-LR” OR “MC-YR” OR “MC-RR” OR “MC-AR” OR “MC-LF” OR “MC-LW” OR “MC-FR” OR “MC-deLR” OR “MC-deRR” OR “MC-deLW” OR “MC-deLF” OR “MC-(O)Y” OR “MC-m[Glu(OCH3)]-LR”)

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Number of records retrieved	**5**
Information source	Records coming from other search
Number of records retrieved	**353**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**1066**
Duplicates	431
Number of records for primary screening	**633**
Deleted due to exclusion criteria (1st screening)	**233**
Number of records for secondary screening	**400**
Further excluded during secondary Screening	**Total: 233** Low quality: 27 Off topics: 172 Other searches: 34
Reviews	43 Not eligible for data extraction
Number of records eligible for data extraction	**124**
Number of extracted records	**116**
Not extracted	8 (No paper available)

Table 20: Information source form for **Nodularins** Search 3

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Maura Manganelli
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 3: Environmental factors affecting cyanotoxin toxicity** **Nodularins**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEYTI (("toxin production" OR (toxicity AND

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	"environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR AB (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR KW (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) AND TI (nodularin*) OR AB (nodularin*) OR KW (nodularin*)
Number of records retrieved	**23**
Information source	**PubMed** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	ALL FIELDS(("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication)) AND (nodularin*))
Number of records retrieved	**23**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	14/05/2015
Search terms	TOPIC, from all data bases(("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication)) AND (nodularin*))
Number of records retrieved	**39**
Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY TI (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR AB (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR DE (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) AND TI (nodularin*) OR AB (nodularin*) OR DE (nodularin*)
Number of records retrieved	**3**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY TI (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR AB (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR KW (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) AND TI (nodularin*) OR AB (nodularin*) OR KW (nodularin*)
Number of records retrieved	**2**
Information source	Records coming from other search

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Number of records retrieved	**28**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**118**
Duplicates	51
Number of records for primary screening	**67**
Deleted due to exclusion criteria (1st screening)	**37**
Number of records for secondary screening	**30**
Further excluded during secondary Screening	**Total: 4**
Reviews	**14** Not eligible for data extraction
Number of records potentially eligible for data extraction	**12**
Number of extracted records	**12**

Table 21: Information source form for **Anatoxins** Search 3

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Maura Manganelli
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 3: Environmental factors affecting cyanotoxin toxicity** **Anatoxins**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015

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Search terms	TITLE-ABS-KEYTI ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) OR AB ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) OR KW ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (TI (anatoxin* OR homoanatoxin*) OR AB (anatoxin* OR homoanatoxin*) OR KW (anatoxin* OR homoanatoxin*))
Number of records retrieved	**29**
Information source	**PubMed** (performed by ISS)
Last Date accessed	27/05/2015
Step 1: Search terms	ALL FIELDS((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (anatoxin* OR homoanatoxin*)
Number of records retrieved	**23**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TOPIC, from all data bases((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (anatoxin* OR homoanatoxin*)
Number of records retrieved	**37**
Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY TI ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) OR AB ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) OR DE ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (TI (anatoxin* OR homoanatoxin*) OR AB (anatoxin* OR homoanatoxin*) OR DE (anatoxin* OR homoanatoxin*))
Number of records retrieved	**1**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY TI ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) OR AB ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) OR KW ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (TI (anatoxin* OR homoanatoxin*) OR AB (anatoxin* OR homoanatoxin*) OR KW (anatoxin* OR homoanatoxin*))
Number of records retrieved	**0**
Information source	Records coming from other searches

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Number of records retrieved	**28**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**118**
Duplicates	49
Number of records for primary screening	**69**
Deleted due to exclusion criteria (1st screening)	**43**
Number of records for secondary screening	**26**
Further excluded during secondary Screening	**4**
Reviews	**17** Not eligible for data extraction
Number of records potentially eligible for data extraction	**5**
Number of extracted records	**5**

Table 22: Information source form for **Cyndrospermoposin** Search 3

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Maura Manganelli
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 3: Environmental factors affecting cyanotoxin toxicity** **Cylindrospermopsin**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEYTI (("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) OR AB (("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) OR KW (("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) AND (TI (cylindrospermopsin*) OR AB (cylindrospermopsin*) OR KW (cylindrospermopsin*))
Number of records retrieved	**33**

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Information source	**PubMed** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	ALL FIELDS((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (cylindrospermopsin*)
Number of records retrieved	**25**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TOPIC, from all data bases((“toxin production” OR (toxicity AND “environmental factors”) OR (toxicity AND “climate changes”) OR (toxicity AND eutrophication)) AND (cylindrospermopsin*))
Number of records retrieved	**39**
Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY TI ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) OR AB ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) OR DE ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (TI (cylindrospermopsin*) OR AB (cylindrospermopsin*) OR DE (cylindrospermopsin*))
Number of records retrieved	**3**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY TI ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) OR AB ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) OR KW ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (TI (anatoxin* OR homoanatoxin*) OR AB (anatoxin* OR homoanatoxin*) OR KW (anatoxin* OR homoanatoxin*))
Number of records retrieved	**0**
Information source	Records coming from other searches
Number of records retrieved	**56**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**156**
Duplicates	52
Number of records for primary screening	**104**

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Deleted due to exclusion criteria (1st screening)	54
Number of records for secondary screening	**50**
Further excluded during secondary Screening	20
Reviews	19 Not eligible for data extraction
Number of records potentially eligible for data extraction	**11**
Number of extracted records	**11**

Table 23: Information source form for **Saxitoxins** Search 3

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Maura Manganelli
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 3: Environmental factors affecting cyanotoxin toxicity** **Saxitoxins**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) **Title OR** ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) **Abstract OR** ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) **Keywords AND** (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Title OR** (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Abstract OR** (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Keywords**
Number of records retrieved	**26**
Information source	**PubMed** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	ALL FIELDS((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (carbamoyl

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	saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*)
Number of records retrieved	**14**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TOPIC, from all data bases((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*)
Number of records retrieved	**29**
Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) **Title OR** ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) **Abstract OR** ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) **Descriptors AND** (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Title OR** (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Abstract OR** (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) **Descriptors**
Number of records retrieved	**0**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Title OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Abstract OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Keywords AND(carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) Title OR (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) Abstract OR (carbamoyl saxitoxin OR decarbamoylsaxitoxin OR sulfocarbamoyl saxitoxin OR *saxitoxin*) Keywords
Number of records retrieved	**1**
Information source	Records coming from other searches
Number of records retrieved	**19**

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Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	89
Duplicates	33
Number of records for primary screening	**56**
Deleted due to exclusion criteria (1st screening)	46
Number of records for secondary screening	**10**
Further excluded during secondary Screening	0
Reviews	5 Not eligible for data extraction
Number of records potentially eligible for data extraction	**5**
Number of extracted records:	**5**

Table 24: Information source form for **LPS** Search 3

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Maura Manganelli
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 3: Environmental factors affecting cyanotoxin toxicity** **LPS**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) **Title OR** ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) **Abstract OR** ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) **Keywords AND** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Title OR** ((lipopolysaccharide OR LPS) AND cyanobacteria)**Abstract OR** ((lipopolysaccharide OR LPS) AND

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	cyanobacteria) **Keywords**
Number of records retrieved	**6**
Information source	**PubMed** (performed by ISS)
Last Date accessed	25/05/2015
Step 1: Search terms	ALL FIELDS((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND ((lipopolysaccharide OR LPS) AND cyanobacteria)
Number of records retrieved	**3**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TOPIC, from all data bases((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND ((lipopolysaccharide OR LPS) AND cyanobacteria)
Number of records retrieved	**4**
Information source	**Agricola** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TITLE-ABS-KEY((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) **Title OR** ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) **Abstract OR** ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) **Descriptors AND** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Title OR** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Abstract OR** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Descriptors**
Number of records retrieved	**0**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Title OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Abstract OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Keywords AND((lipopolysaccharide OR LPS) AND cyanobacteria) Title OR ((lipopolysaccharide OR LPS) AND cyanobacteria) Abstract OR ((lipopolysaccharide OR LPS) AND cyanobacteria) Keywords
Number of records retrieved	**0**
Information source	Records coming from other searches
Number of records retrieved	**1**

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Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	14
Duplicates	4
Number of records for primary screening	**10**
Deleted due to exclusion criteria (1st screening)	**8**
Number of records for secondary screening	**2**
Further excluded during secondary Screening	**0**
Reviews	**2** Not eligible for data extraction
Number of records potentially eligible for data extraction	**0**

Table 25: Information source form for **BMAA** Search 3

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Maura Manganelli
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 3: Environmental factors affecting cyanotoxin toxicity** **BMAA**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Title OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Abstract OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Keywords AND(BMAA OR beta-Methylamino-L-alanine) Title OR (BMAA OR beta-Methylamino-L-alanine) Abstract OR (BMAA OR beta-Methylamino-L-alanine) Keywords
Number of records retrieved	**5**

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Information source	**PubMed** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	ALL FIELDS(("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) AND (BMAA OR beta-Methylamino-L-alanine)
Number of records retrieved	**10**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TOPIC, from all data bases(("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) AND (BMAA OR beta-Methylamino-L-alanine) AND (BMAA OR beta-Methylamino-L-alanine)
Number of records retrieved	**8**
Information source	**Agricola** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TITLE-ABS-KEY(("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) Title OR ("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) Abstract OR ("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) Descriptors AND(BMAA OR beta-Methylamino-L-alanine) Title OR (BMAA OR beta-Methylamino-L-alanine) Abstract OR (BMAA OR beta-Methylamino-L-alanine) Descriptors
Number of records retrieved	**0**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY ("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) Title OR ("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) Abstract OR ("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) Keywords AND(BMAA OR beta-Methylamino-L-alanine) Title OR (BMAA OR beta-Methylamino-L-alanine) Abstract OR (BMAA OR beta-Methylamino-L-alanine) Keywords
Number of records retrieved	**0**
Information source	Records coming from other searches
Number of records retrieved	**1**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	24

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Duplicates	11
Number of records for primary screening	**13**
Deleted due to exclusion criteria (1st screening)	10
Number of records for secondary screening	**3**
Further excluded during secondary Screening	0
Reviews	2 Not eligible for data extraction
Number of records potentially eligible for data extraction	**1**
Number of extracted records	**1**

Table 26: Information source form for **Aplysiatoxin** Search 3

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Maura Manganelli
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 3: Environmental factors affecting cyanotoxin toxicity** **Aplysiatoxin**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Title OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Abstract OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Keywords AND(aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Title OR (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Abstract OR (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Keywords
Number of records retrieved	**3**

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Information source	**PubMed** (performed by ISS)
Last Date accessed	23/05/2015
Search terms	ALL FIELDS((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*)
Number of records retrieved	**0**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	23/05/2015
Search terms	TOPIC, from all data bases((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*)
Number of records retrieved	**0**
Information source	**Agricola** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TITLE-ABS-KEY((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Title OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Abstract OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Descriptors AND(aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Title OR (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Abstract OR (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Descriptors
Number of records retrieved	**0**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Title OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Abstract OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Keywords AND(aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Title OR (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Abstract OR (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Keywords
Number of records retrieved	**0**

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Information source	Records coming from other searches
Number of records retrieved	**0**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**3**
Duplicates	0
Number of records for primary screening	**3**
Deleted due to exclusion criteria (1st screening)	2
Number of records for secondary screening	**1**
Further excluded during secondary Screening	0
Reviews	1 Not eligible for data extraction
Number of records potentially eligible for data extraction	**0**

Table 27: Information source form for **Lyngbyatoxin** Search 3

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Maura Manganelli
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 3: Environmental factors affecting cyanotoxin toxicity** **Lyngbyatoxin**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Title OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Abstract OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND

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	(toxicity)) Keywords AND(lyngbyatoxin*) **Title OR**(lyngbyatoxin*)**Abstract OR**(lyngbyatoxin*) **Keywords**
Number of records retrieved	**3**
Information source	**PubMed** (performed by ISS)
Last Date accessed	23/05/2015
Search terms	ALL FIELDS((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (lyngbyatoxin*)
Number of records retrieved	**0**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	23/05/2015
Search terms	TOPIC, from all data bases((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity))AND (lyngbyatoxin*)
Number of records retrieved	**0**
Information source	**Agricola** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TITLE-ABS-KEY((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Title OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Abstract OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Descriptors AND(lyngbyatoxin*) Title OR (lyngbyatoxin*) Abstract OR (lyngbyatoxin*) Descriptors
Number of records retrieved	**0**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Title OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Abstract OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Keywords AND(lyngbyatoxin*) Title OR (lyngbyatoxin*) Abstract OR (lyngbyatoxin*) Keywords
Number of records retrieved	**0**
Information source	Records coming from other searches
Number of records retrieved	**0**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**

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Number of records retrieved	3
Duplicates	0
Number of records for primary screening	**3**
Deleted due to exclusion criteria (1st screening)	**3**
Number of records for secondary screening	**0**
Number of records eligible for data extraction	**0**

Table 28: Information source form for **Palytoxin** Search 3

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Maura Manganelli
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 3: Environmental factors affecting cyanotoxin toxicity** **Palytoxin**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Title OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Abstract OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Keywords AND(palytoxin* OR PlTX OR ostreocin*) Title OR (palytoxin* OR PlTX OR ostreocin*) Abstract OR (palytoxin* OR PlTX OR ostreocin*) Keywords
Number of records retrieved	**8**
Information source	**PubMed** (performed by ISS)
Last Date accessed	23/05/2015
Search terms	ALL FIELDS((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (palytoxin* OR PlTX OR ostreocin*)
Number of records retrieved	**8**

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Information source	**Web of Science** (performed by ISS)
Last Date accessed	23/05/2015
Search terms	TOPIC, from all data bases((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (palytoxin* OR PlTX OR ostreocin*)
Number of records retrieved	**8**
Information source	**Agricola** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TITLE-ABS-KEY((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Title OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Abstract OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Descriptors AND(palytoxin* OR PlTX OR ostreocin*) Title OR (palytoxin* OR PlTX OR ostreocin*) Abstract OR (palytoxin* OR PlTX OR ostreocin*) Descriptors
Number of records retrieved	**1**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Title OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Abstract OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Keywords AND(palytoxin* OR PlTX OR ostreocin*) Title OR (palytoxin* OR PlTX OR ostreocin*) Abstract OR (palytoxin* OR PlTX OR ostreocin*) Keywords
Number of records retrieved	**0**
Information source	Records coming from other searches
Number of records retrieved	**12**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	37
Duplicates	16
Number of records for primary screening	**21**
Deleted due to exclusion criteria (1st screening)	**21**
Number of records for secondary screening	**0**

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Further excluded during secondary Screening	**0**
Reviews	Not eligible for data extraction
Number of records eligible for data extraction	**0**

Table 29: Information source form for **Ciguatoxin** Search 3

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Maura Manganelli
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 3: Environmental factors affecting cyanotoxin toxicity** **Ciguatoxin**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Title OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Abstract OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Keywords AND(*ciguatoxin* OR (CTX AND cyanobacteria)) Title OR (*ciguatoxin* OR (CTX AND cyanobacteria)) Abstract OR (*ciguatoxin* OR (CTX AND cyanobacteria)) Keywords
Number of records retrieved	**13**
Information source	**PubMed** (performed by ISS)
Last Date accessed	23/05/2015
Search terms	ALL FIELDSALL FIELDS ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) AND (*ciguatoxin* OR (CTX AND cyanobacteria))
Number of records retrieved	**1**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	23/05/2015
Search terms	TOPIC, from all data bases((“toxin production” OR “environmental

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	factors" OR "climate changes" OR eutrophication) AND (toxicity)) AND (*ciguatoxin* OR (CTX AND cyanobacteria))
Number of records retrieved	**2**
Information source	**Agricola** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TITLE-ABS-KEY(("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) Title OR (("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) Abstract OR (("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) Descriptors AND(*ciguatoxin* OR (CTX AND cyanobacteria)) Title OR (*ciguatoxin* OR (CTX AND cyanobacteria)) Abstract OR (*ciguatoxin* OR (CTX AND cyanobacteria)) Descriptors
Number of records retrieved	**1**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY (("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) Title OR (("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) Abstract OR (("toxin production" OR "environmental factors" OR "climate changes" OR eutrophication) AND (toxicity)) Keywords AND(*ciguatoxin* OR (CTX AND cyanobacteria)) Title OR (*ciguatoxin* OR (CTX AND cyanobacteria)) Abstract OR (*ciguatoxin* OR (CTX AND cyanobacteria)) Keywords
Number of records retrieved	**0**
Information source	Records coming from other searches
Number of records retrieved	**2**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	19
Duplicates	4
Number of records for primary screening	**15**
Deleted due to exclusion criteria (1st screening)	**12**
Number of records for secondary screening	**3**
Further excluded during secondary Screening	**0**
Reviews	**3** Not eligible for data extraction
Number of records	**0**

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eligible for data extraction	

Table 30: Information source form for **Cyanobacterial extracts** Search 3

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Maura Manganelli
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 3: Environmental factors affecting cyanotoxin toxicity** **Cyanobacterial extracts**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: TI (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR AB (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR KW (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) AND (TI (cyanobacteria* extract) OR AB (cyanobacteria* extract) OR KW (cyanobacteria* extract))
Number of records retrieved	**12**
Information source	**PubMed** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	ALL FIELDS ("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication)) AND ("cyanobacteria/l extract")
Number of records retrieved	**6**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	**TOPIC:**("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication)) AND ("cyanobacteria/l extract")
Number of records retrieved	**7**

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Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: TI (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR AB (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR DE (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) AND (TI (cyanobacteria* extract) OR AB (cyanobacteria* extract) OR DE (cyanobacteria* extract))
Number of records retrieved	**1**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: TI (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR AB (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR KW (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) AND (TI (cyanobacteria* extract) OR AB (cyanobacteria* extract) OR KW (cyanobacteria* extract))
Number of records retrieved	**0**
Information source	Records coming from other searches
Number of records retrieved	6
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	32
Duplicates	12
Number of records for primary screening	**20**
Deleted due to exclusion criteria (1st screening)	**20**
Number of records for secondary screening	**0**
Number of records eligible for data extraction	**0**

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Table 31: Information source form for **Cyanotoxins** Search 3

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Maura Manganelli/Franca M. Buratti
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 3: Environmental factors affecting cyanotoxin toxicity** **Cyanotoxins NOT**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEYWORDS: TI (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR AB (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR KW (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) AND (TI (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*) OR AB (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*) OR KW (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*))
Number of records retrieved	**6**
Information source	**PubMed** (performed by ISS)
Last Date accessed	29/05/2015
Search terms	**ALL FIELDS**("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication)) AND (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin*

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	NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*)
Number of records retrieved	**2**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	**TOPIC:**("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication)) **AND**(cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*))
Number of records retrieved	**5**
Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: TI (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR AB (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR DE (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) **AND** (TI (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*) OR AB (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*) OR DE (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*))
Number of records retrieved	**2**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: TI (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) OR AB (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate

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	changes") OR (toxicity AND eutrophication))) OR KW (("toxin production" OR (toxicity AND "environmental factors") OR (toxicity AND "climate changes") OR (toxicity AND eutrophication))) AND (TI (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*) OR AB (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*) OR KW (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*))
Number of records retrieved	**1**
Information source	Records coming from other searches
Number of records retrieved	29
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	45
Duplicates	11
Number of records for primary screening	**34**
Deleted due to exclusion criteria (1st screening)	**21**
Number of records for secondary screening	**13**
Deleted due to exclusion criteria (2nd screening)	**2**
Reviews	**11** Not eligible for data extraction
Number of records potentially eligible for data extraction	**0**

A.7 Toxicity following combined exposure to mixture of cyanotoxins and other chemicals

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Table 32: Information source form for **Microcystins** Search 4

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Franca M. Buratti/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 4: Combined effects due to mixture of cyanotoxins and/or other xenobiotics** **Microcystins**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) AND (microcystin* OR “MC-LR” OR “MC-YR” OR “MC-RR” OR “MC-AR” OR “MC-LF” OR “MC-LW” OR “MC-FR” OR “MC-deLR” OR “MC-deRR” OR “MC-deLW” OR “MC-deLF” OR “MC-(O)Y” OR “MC-m[Glu(OCH_3)]-LR”)
Number of records retrieved	**392**
Information source	**PubMed** (performed by ISS)
Last Date accessed	21/05/2015
Search terms	ALL FIELDS((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) AND (microcystin*)
Number of records retrieved	**284**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	21/05/2015
Search terms	TOPIC, from all data bases((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) AND (microcystin* OR “MC-LR” OR “MC-YR” OR “MC-RR” OR “MC-AR” OR “MC-LF” OR “MC-LW” OR “MC-FR” OR “MC-deLR” OR “MC-deRR” OR “MC-deLW” OR “MC-deLF” OR “MC-(O)Y” OR “MC-m[Glu(OCH_3)]-LR”)
Number of records retrieved	**503**
Information source	**Agricola** (performed by ISS)

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Last Date accessed	28/05/2015
Search terms	TITLE-ABS-KEY (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) AND (microcystin* OR "MC-LR" OR "MC-YR" OR "MC-RR" OR "MC-AR" OR "MC-LF" OR "MC-LW" OR "MC-FR" OR "MC-deLR" OR "MC-deRR" OR "MC-deLW" OR "MC-deLF" OR "MC-(O)Y" OR "MC-m[Glu(OCH3)]-LR")
Number of records retrieved	**28**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	21/05/2015
Search terms	TITLE-ABS-KEY (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) AND (microcystin* OR "MC-LR" OR "MC-YR" OR "MC-RR" OR "MC-AR" OR "MC-LF" OR "MC-LW" OR "MC-FR" OR "MC-deLR" OR "MC-deRR" OR "MC-deLW" OR "MC-deLF" OR "MC-(O)Y" OR "MC-m[Glu(OCH_3)]-LR")
Number of records retrieved	**21**
Information source	Records coming from other search
Number of records retrieved	**75**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**1303**
Duplicates	**611**
Number of records for primary screening	**692**
Deleted due to exclusion criteria (1st screening)	**Total: 322** Language 7 Off topic (e.g. occurrence, methods of analysis)197 Other cyanotoxins 20 Plants and incertebrates (no mixture): 98
Number of records for secondary screening	**370**
Further excluded during secondary Screening	**Total: 242** No Mixture: 194 Uncharacterised Extracts: 4 Search 3 :43 Not available: 1
Reviews	**54** Not eligible for data extraction
Number of records eligible for data extraction	**74**
Number of extracted	**5**

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records	
Subdivision of extracted records	
In vivo	5
Subdivision of the records eligible but not extracted due to low priority criteria for data extraction	
Chemoprotectant action	17
Fish	18
In vitro	14
In vivo birds	3
Invertebrated with interactions	6
Treatment/Antidotes	1

Table 33: Information source form for **Nodularins** Search 4

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Maura Manganelli/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 4: Combined effects due to mixture of cyanotoxins and/or other xenobiotics** **Nodularins**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY(TI((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”)) OR AB ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”)) OR KW ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”))) AND (TI (nodularin*) OR AB (nodularin*) OR KW (nodularin*))
Number of records retrieved	**49**
Information source	**PubMed** (performed by ISS)

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Last Date accessed	27/05/2015
Search terms	ALL FIELDS("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (nodularin*)
Number of records retrieved	**82**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TOPIC, from all data bases(("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (nodularin*))
Number of records retrieved	**213**
Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY (TI (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR AB (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR DE (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin"))) AND (TI (nodularin*) OR AB (nodularin*) OR DE (nodularin*))
Number of records retrieved	**12**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY (TI (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR AB (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR KW (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin"))) AND (TI (nodularin*) OR AB (nodularin*) OR KW (nodularin*))
Number of records retrieved	**2**
Information source	Records coming from other search
Number of records retrieved	**0**

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Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**358**
Duplicates	134
Number of records for primary screening	**224**
Deleted due to exclusion criteria (1st screening)	**Total:105** Off topic: 38 Occurrence and methods: 60 Invertebrates and plants: 7
Number of records for secondary screening	**119**
Further excluded during secondary Screening	**Total: 92** Only MC: 56 No mixture: 36
Reviews	24 Not eligible for data extraction
Number of records eligible for data extraction	**3**
Records not extracted due to second/low priority	2
Number of extracted records	**1**

Table 34: Information source form for **Anatoxins** Search 4

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Maura Manganelli/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 4: Combined effects due to mixture of cyanotoxins and/or other xenobiotics** **Anatoxins**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY(TI (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR AB (("concomitant exposure" OR

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	interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR KW (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin"))) AND (TI (anatoxin* OR homoanatoxin*) OR AB (anatoxin* OR homoanatoxin*) OR KW (anatoxin* OR homoanatoxin*))
Number of records retrieved	**41**
Information source	**PubMed** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	ALL FIELDS("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (anatoxin* OR homoanatoxin*)
Number of records retrieved	**97**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TOPIC, from all data bases("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (anatoxin* OR homoanatoxin*)
Number of records retrieved	**178**
Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY (TI (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR AB (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR DE (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin"))) AND (TI (anatoxin* OR homoanatoxin*) OR AB (anatoxin* OR homoanatoxin*) OR DE (anatoxin* OR homoanatoxin*))
Number of records retrieved	**6**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY (TI (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR AB (("concomitant exposure" OR

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	interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR KW (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin"))) AND (TI (anatoxin* OR homoanatoxin*) OR AB (anatoxin* OR homoanatoxin*) OR KW (anatoxin* OR homoanatoxin*))
Number of records retrieved	**8**
Information source	Records coming from other searches
Number of records retrieved	**0**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**330**
Duplicates	129
Number of records for primary screening	**201**
Deleted due to exclusion criteria (1st screening)	**Total: 133** Language 1 Off Topic (other toxins): 89 Occurrence, methods other searches: 33 Invertebrates and plants 10
Number of records for secondary screening	**68**
Further excluded during secondary Screening	**Total: 45** No Mixture: 31 Toxin used as a probe: 12
Reviews	**22** Not eligible for data extraction
Number of records eligible for data extraction	**1**
Records not extracted due to second/low priority	**1**
Number of extracted records	**0**

Table 35: Information source form for **Cyndrospermoposin** Search 4

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04

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INFORMATION SCIENTIST	Maura Manganelli/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 4: Combined effects due to mixture of cyanotoxins and/or other xenobiotics** **Cylindrospermopsin**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY (TI((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”)) OR AB ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”)) OR KW ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”))) AND (TI (cylindrospermopsin*) OR AB (cylindrospermopsin*) OR KW (cylindrospermopsin*))
Number of records retrieved	**47**
Information source	**PubMed** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	ALL FIELDS(“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (cylindrospermopsin*)
Number of records retrieved	**60**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TOPIC, from all data bases((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (cylindrospermopsin*))
Number of records retrieved	**134**
Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY (TI ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”)) OR AB ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction

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	OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR DE (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin"))) AND (TI (cylindrospermopsin*) OR AB (cylindrospermopsin*) OR DE (cylindrospermopsin*))
Number of records retrieved	**12**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY(TI (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR AB (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR KW (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin"))) AND (TI (cylindrospermopsin*) OR AB (cylindrospermopsin*) OR KW (cylindrospermopsin*))
Number of records retrieved	**6**
Information source	Records coming from other searches
Number of records retrieved	**0**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**259**
Duplicates	107
Number of records for primary screening	**152**
Deleted due to exclusion criteria (1st screening)	**Total: 59** Invertebrates and plants (no mixture): 16 Occurrence, methods: 22 Off topic 21
Number of records for secondary screening	**93**
Further excluded during secondary Screening	**Total: 61**
Reviews	30 Not eligible for data extraction
Number of records eligible for data extraction	**2**
Records not extracted due to	**2**

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second/low priority	
Number of extracted records	**0**

Table 36: Information source form for **Saxitoxins** Search 4

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 4: Combined effects due to mixture of cyanotoxins and/or other xenobiotics** **Saxitoxins**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY(("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Title OR** (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Abstract OR** (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Keywords AND** (*saxitoxin*) **Title OR** (*saxitoxin*) **Abstract OR** (*saxitoxin*) **Keywords**
Number of records retrieved	**33**
Information source	**PubMed** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	ALL FIELDS(("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) AND (*saxitoxin*)
Number of records retrieved	**19**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	28/05/2015

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Search terms	TOPIC, from all data bases((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) AND (*saxitoxin*)
Number of records retrieved	**34**
Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) **Title OR** ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) **Abstract OR** ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) **Descriptors AND** (*saxitoxin*) **Title OR** (*saxitoxin*) **Abstract OR** (*saxitoxin*) **Descriptors**
Number of records retrieved	**7**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) **Title OR** ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) **Abstract OR** ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) **Keywords AND** (*saxitoxin*) **Title OR** (*saxitoxin*) **Abstract OR** (*saxitoxin*) **Keywords**
Number of records retrieved	**2**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**95**
Duplicates	47
Number of records for primary screening	**49**

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Deleted due to exclusion criteria (1st screening)	**47**
Number of records for secondary screening	**2**
Further excluded during secondary Screening	**0**
Number of records eligible for data extraction	**2**
Number of extracted records	**2**

Table 37: Information source form for **LPS** Search 4

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 4: Combined effects due to mixture of cyanotoxins and/or other xenobiotics** **LPS**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY(("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Title OR** (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Abstract OR** (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Keywords AND** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Title OR** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Abstract OR** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Keywords**
Number of records retrieved	**11**
Information source	**PubMed** (performed by ISS)

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Last Date accessed	25/05/2015
Search terms	ALL FIELDS(("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) AND ((lipopolysaccharide OR LPS) AND cyanobacteria)
Number of records retrieved	**5**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TOPIC, from all data bases((lipopolysaccharide OR LPS) AND cyanobacteria) **Title OR** ((lipopolysaccharide OR LPS) AND cyanobacteria)**Abstract OR** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Keywords**
Number of records retrieved	**10**
Information source	**Agricola** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TITLE-ABS-KEY(("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) Title OR (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) Abstract OR (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) Descriptors **AND** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Title OR** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Abstract OR** ((lipopolysaccharide OR LPS) AND cyanobacteria) **Descriptors**
Number of records retrieved	**1**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) Title OR (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) Abstract OR (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) Keywords

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	AND((lipopolysaccharide OR LPS) AND cyanobacteria) Title OR ((lipopolysaccharide OR LPS) AND cyanobacteria) Abstract OR ((lipopolysaccharide OR LPS) AND cyanobacteria) Keywords
Number of records retrieved	**0**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**27**
Duplicates	8
Number of records for primary screening	**19**
Deleted due to exclusion criteria (1st screening)	**17**
Number of records for secondary screening	**2**
Further excluded during secondary Screening	**0**
Number of records eligible for data extraction	**2**
Records not extracted due to second/low priority	2
Number of extracted records	**0**

Table 38: Information source form for **BMAA** Search 4

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 4: Combined effects due to mixture of cyanotoxins and/or other xenobiotics** **BMAA**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY(("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy

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	metal" OR "other toxin") AND (toxicity)) Title OR (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) Abstract OR (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) Keywords AND(BMAA OR beta-Methylamino-L-alanine) Title OR (BMAA OR beta-Methylamino-L-alanine) Abstract OR (BMAA OR beta-Methylamino-L-alanine) Keywords
Number of records retrieved	**13**
Information source	**PubMed** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	ALL FIELDS(("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) AND (BMAA OR beta-Methylamino-L-alanine)
Number of records retrieved	**9**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TOPIC, from all data bases(("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) AND (BMAA OR beta-Methylamino-L-alanine)
Number of records retrieved	**13**
Information source	**Agricola** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TITLE-ABS-KEY(("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) Title OR (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) Abstract OR (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) Descriptors AND(BMAA OR beta-Methylamino-L-alanine) Title OR (BMAA OR beta-Methylamino-L-alanine) Abstract OR (BMAA OR beta-Methylamino-L-alanine) Descriptors

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Number of records retrieved	**1**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Title OR ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Abstract OR ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Keywords AND(BMAA OR beta-Methylamino-L-alanine) Title OR (BMAA OR beta-Methylamino-L-alanine) Abstract OR (BMAA OR beta-Methylamino-L-alanine) Keywords
Number of records retrieved	**0**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**36**
Duplicates	17
Number of records for primary screening	**18**
Deleted due to exclusion criteria (1st screening)	**16**
Number of records for secondary screening	**2**
Further excluded during secondary Screening	**0**
Number of records eligible for data extraction	**2**
Records not extracted due to second/low priority	2
Number of extracted records	**0**

Table 39: Information source form for **Aplysiatoxin** Search 4

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food

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EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 4: Combined effects due to mixture of cyanotoxins and/or other xenobiotics** **Aplysiatoxin**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Title OR ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Abstract OR ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Keywords AND(aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Title OR (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Abstract OR (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Keywords
Number of records retrieved	**2**
Information source	**PubMed** (performed by ISS)
Last Date accessed	28/05/2015
Search terms	ALL FIELDS((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) AND (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*)
Number of records retrieved	**8**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	28/05/2015
Search terms	TOPIC, from all data bases((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) AND (aplysiatoxin*

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	OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*)
Number of records retrieved	**13**
Information source	**Agricola** (performed by ISS)
Last Date accessed	28/05/2015
Search terms	TITLE-ABS-KEY((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Title OR ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Abstract OR ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Descriptors AND(aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Title OR (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Abstract OR (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Descriptors
Number of records retrieved	**0**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	28/05/2015
Search terms	TITLE-ABS-KEY ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Title OR ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Abstract OR ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Keywords AND(aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Title OR (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Abstract OR (aplysiatoxin* OR anhydroaplysiatoxin* OR debromoaplysiatoxin* OR anhydrodebromoaplysiatoxin*) Keywords
Number of records retrieved	**0**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed +**

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	Scopus + other Sources
Number of records retrieved	**23**
Duplicates	9
Number of records for primary screening	**14**
Deleted due to exclusion criteria (1st screening)	**13**
Number of records for secondary screening	**1**
Further excluded during secondary Screening	**0**
Number of records eligible for data extraction	**1**
Number of extracted records	**1**

Table 40: Information source form for **Lyngbyatoxin** Search 4

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 4: Combined effects due to mixture of cyanotoxins and/or other xenobiotics** **Lyngbyatoxin**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Title OR ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Abstract OR ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Keywords AND

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	(lyngbyatoxin*) **Title OR** (lyngbyatoxin*) **Abstract OR** (lyngbyatoxin*) **Keywords**
Number of records retrieved	**3**
Information source	**PubMed** (performed by ISS)
Last Date accessed	23/05/2015
Search terms	ALL FIELDS((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) AND (lyngbyatoxin*)
Number of records retrieved	**6**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	23/05/2015
Search terms	TOPIC, from all data bases((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) AND (lyngbyatoxin*)
Number of records retrieved	**8**
Information source	**Agricola** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TITLE-ABS-KEY((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) **Title OR** ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) **Abstract OR** ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) **Descriptors**AND(lyngbyatoxin*) Title OR (lyngbyatoxin*) Abstract OR (lyngbyatoxin*) Descriptors
Number of records retrieved	**0**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	28/05/2015
Search terms	TITLE-ABS-KEY ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Title OR ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) Abstract OR ((“concomitant exposure” OR interaction OR

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	synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) Keywords AND(lyngbyatoxin*) Title OR (lyngbyatoxin*) Abstract OR (lyngbyatoxin*) Keywords
Number of records retrieved	**0**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**17**
Duplicates	7
Number of records for primary screening	**10**
Deleted due to exclusion criteria (1st screening)	**9**
Number of records for secondary screening	**1**
Further excluded during secondary Screening	**0**
Number of records eligible for data extraction	**1**
Number of extracted records	**1**

Table 41: Information source form for **Palytoxin** Search 4

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 4: Combined effects due to mixture of cyanotoxins and/or other xenobiotics** **Palytoxin**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY(("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy

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	metal" OR "other toxin") AND (toxicity)) **Title OR** (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Abstract OR** (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Keywords** AND (palytoxin* OR PlTX OR ostreocin*) Title OR (palytoxin* OR PlTX OR ostreocin*) Abstract OR (palytoxin* OR PlTX OR ostreocin*) Keywords
Number of records retrieved	**8**
Information source	**PubMed** (performed by ISS)
Last Date accessed	23/05/2015
Search terms	ALL FIELDS(("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) AND (palytoxin* OR PlTX OR ostreocin*)
Number of records retrieved	**8**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	23/05/2015
Search terms	TOPIC, from all data bases(("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) AND (palytoxin* OR PlTX OR ostreocin*)
Number of records retrieved	**13**
Information source	**Agricola** (performed by ISS)
Last Date accessed	25/05/2015
Search terms	TITLE-ABS-KEY(("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Title OR** (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Abstract OR** (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Descriptors**AND(palytoxin* OR PlTX OR ostreocin*) Title OR (palytoxin* OR PlTX OR ostreocin*) Abstract OR (palytoxin* OR PlTX OR ostreocin*) Descriptors
Number of records retrieved	**1**

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Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEY ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Title OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Abstract OR ((“toxin production” OR “environmental factors” OR “climate changes” OR eutrophication) AND (toxicity)) Keywords AND(palytoxin* OR PlTX OR ostreocin*) Title OR (palytoxin* OR PlTX OR ostreocin*) Abstract OR (palytoxin* OR PlTX OR ostreocin*) Keywords
Number of records retrieved	**0**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**30**
Duplicates	13
Number of records for primary screening	**16**
Deleted due to exclusion criteria (1st screening)	**11**
Number of records for secondary screening	**5**
Further excluded during secondary Screening	**0**
Number of records eligible for data extraction	**5**
Records not extracted due to second/low priority	**2**
Number of extracted records	**3**

Table 42: Information source form for **Ciguatoxin** Search 4

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Susanna Vichi/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 4: Combined effects due to mixture of cyanotoxins and/or other xenobiotics**

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	Ciguatoxin
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEY((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) **Title OR** ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) **Abstract OR** ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) **Keywords** AND (*ciguatoxin* OR (CTX AND cyanobacteria)) Title OR (*ciguatoxin* OR (CTX AND cyanobacteria)) Abstract OR (*ciguatoxin* OR (CTX AND cyanobacteria)) Keywords
Number of records retrieved	**9**
Information source	**PubMed** (performed by ISS)
Last Date accessed	23/05/2015
Search terms	ALL FIELDS((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) AND (*ciguatoxin* OR (CTX AND cyanobacteria))
Number of records retrieved	**11**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	23/05/2015
Search terms	TOPIC, from all data bases((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) AND (*ciguatoxin* OR (CTX AND cyanobacteria))
Number of records retrieved	**14**
Information source	**Agricola** (performed by ISS)
Last Date accessed	28/05/2015
Search terms	TITLE-ABS-KEY((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (toxicity)) **Title OR** ((“concomitant exposure” OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR

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	"water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Abstract OR** (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Descriptors** AND (*ciguatoxin* OR (CTX AND cyanobacteria)) Title OR (*ciguatoxin* OR (CTX AND cyanobacteria)) Abstract OR (*ciguatoxin* OR (CTX AND cyanobacteria)) Descriptors
Number of records retrieved	**1**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	28/05/2015
Search terms	TITLE-ABS-KEY (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Title OR** (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Abstract OR** (("concomitant exposure" OR interaction OR synergism OR additivity OR antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND (toxicity)) **Keywords** AND (*ciguatoxin* OR (CTX AND cyanobacteria)) Title OR (*ciguatoxin* OR (CTX AND cyanobacteria)) Abstract OR (*ciguatoxin* OR (CTX AND cyanobacteria)) Keywords
Number of records retrieved	**1**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**36**
Duplicates	15
Number of records for primary screening	**18**
Deleted due to exclusion criteria (1st screening)	**16**
Number of records for secondary screening	**2**
Further excluded during secondary Screening	**0**
Number of records eligible for data extraction	**2**
Records not extracted due to second/low priority	**2**
Number of extracted	**0**

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records	

Table 43: Information source form for **Cyanobacterial extracts** Search 4

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Maura Manganelli/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 4: Combined effects due to mixture of cyanotoxins and/or other xenobiotics** **Cyanobacterial extracts**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: (TI((“concomitant exposure” OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”)) OR AB ((“concomitant exposure” OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”)) OR KW ((“concomitant exposure” OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”))) AND (TI (cyanobacteria* extract) OR AB (cyanobacteria* extract) OR KW (cyanobacteria* extract))
Number of records retrieved	**22**
Information source	**PubMed** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	ALL FIELDS (“concomitant exposure” OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR “water contaminant” OR “heavy metal” OR “other toxin”) AND (“cyanobacteria/l extract”)
Number of records retrieved	**10**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	**TOPIC:**(“concomitant exposure” OR interaction OR synergism OR

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	additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") AND ("cyanobacteria/l extract")
Number of records retrieved	**29**
Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: (TI (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR AB (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR DE (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin"))) AND (TI (cyanobacteria* extract) OR AB (cyanobacteria* extract) OR DE (cyanobacteria* extract))
Number of records retrieved	**8**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: (TI (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR AB (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR KW (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin"))) AND (TI (cyanobacteria* extract) OR AB (cyanobacteria* extract) OR KW (cyanobacteria* extract))
Number of records retrieved	**2**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**71**
Duplicates	23
Number of records for primary screening	**48**
Deleted due to exclusion criteria (1st screening)	**42**
Number of records for	**6**

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secondary screening	
Reviews	1 Not eligible for data extraction
Number of records eligible for data extraction	**5**
Records not extracted due to second/low priority	**5**
Number of extracted records	**0**

Table 44: Information source form for **Cyanotoxins** Search 4

EFSA SYSTEMATIC REVIEW	Cyanobacteria toxins in food
EFSA REFERENCE	OC/EFSA/SCER/2014/04
INFORMATION SCIENTIST	Maura Manganelli/Emanuela Testai
STUDY QUESTION	**Objective 2: Cyanotoxin toxicological profile** **Search 4: Combined effects due to mixture of cyanotoxins and/or other xenobiotics** **Cyanotoxins NOT**
Information source	**Scopus** (performed by ANSES)
Last Date accessed	4/06/2015
Search terms	TITLE-ABS-KEYWORDS: (TI (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR AB (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR KW (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin"))) **AND** (TI (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*) OR AB (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*) OR KW (cyanotoxin* NOT microcystin* NOT nodularin* NOT

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	cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*))
Number of records retrieved	**7**
Information source	**PubMed** (performed by ISS)
Last Date accessed	29/05/2015
Search terms	**ALL FIELDS**("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") **AND**(cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*)
Number of records retrieved	**7**
Information source	**Web of Science** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	**TOPIC:**("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin") **AND**(cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*))
Number of records retrieved	**24**
Information source	**Agricola** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: (TI (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR AB (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR DE (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin"))) **AND** (TI (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-

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	Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*) OR AB (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*) OR DE (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*))
Number of records retrieved	**6**
Information source	**Food Science Source** (performed by ISS)
Last Date accessed	27/05/2015
Search terms	TITLE-ABS-KEYWORDS: (TI (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR AB (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin")) OR KW (("concomitant exposure" OR interaction OR synergism OR additivity Or antagonism OR induction OR inhibition OR mixture OR cocktail OR pesticide OR "water contaminant" OR "heavy metal" OR "other toxin"))) **AND** (TI (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*) OR AB (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*) OR KW (cyanotoxin* NOT microcystin* NOT nodularin* NOT cylindrospermopsin* NOT *anatoxin* NOT (BMAA OR beta-N-Methylamino-L-alanine) NOT *saxitoxin* NOT lyngbyatoxin* NOT ((lipopolysaccharide OR LPS) AND cyanobacteria) NOT *aplysiatoxin* NOT (palytoxin OR PLTX OR ostreocin*) NOT *ciguatoxin*))
Number of records retrieved	**12**
Information source	**Web of Science + Agricola + Food Science Source+ PubMed + Scopus + other Sources**
Number of records retrieved	**56**

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Duplicates	18
Number of records for primary screening	**38**
Deleted due to exclusion criteria (1st screening)	**27**
Number of records for secondary screening	**11**
Reviews	9 Not eligible for data extraction
Number of records eligible for data extraction	**2**
Records not extracted due to second/low priority	**2**
Number of extracted records	**0**

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