ANSES Opinion

Request No 2014-SA-0096

The Director General

Maisons-Alfort, 4 August 2017

OPINION

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

on the "risks associated with the consumption of food supplements containing spirulina"

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES's public health mission involves ensuring environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

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

Its opinions are published on its website.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 4 August 2017 shall prevail.

On 11 April 2014, ANSES issued an internal request to conduct an expert appraisal on the following issue: risks associated with the consumption of food supplements containing spirulina.

1. BACKGROUND AND PURPOSE OF THE REQUEST

Between the establishment of its nutrivigilance scheme and February 2017, ANSES received 49 reports of adverse effects likely to be associated with the consumption of food supplements containing spirulina. Ten of these reports contained enough information to be analysed for their causality. Moreover, in 2014, ANSES had revealed a case of allergic reaction (allergic facial angioedema) that occurred after consumption of spirulina. In this context, ANSES issued an internal request to analyse in greater detail the various adverse effects likely to be associated with the consumption of spirulina.

The objective of this opinion is to assess the health risks and not the possible effectiveness or nutritional benefit of food supplements containing spirulina.

2. ORGANISATION OF THE EXPERT APPRAISAL

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

This expert appraisal falls within the scope of the Expert Committee (CES) on "Human Nutrition". ANSES entrusted the expert appraisal to external rapporteurs and to the Working Group (WG) on

"Nutrivigilance". The methodological and scientific aspects of the work were presented to the CES on 27 April 2017. It was adopted by the CES at its meeting on 6 July 2017.

ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal.
The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

The 49 reports of adverse effects likely to be associated with the consumption of food supplements containing spirulina were collected in the framework of the nutrivigilance scheme. These reports were submitted by healthcare professionals, by the French National Agency for Medicines and Health Products Safety (ANSM) and its regional pharmacovigilance centres (CRPV), and by manufacturers of food supplements containing spirulina. Ten reports underwent a causality analysis, carried out using the method developed by ANSES (2011), while the others were regarded as inadmissible (due for example to the dates of consumption being unknown, or the product not being clearly identified).

ANSES asked the poison control centres (CAPs) and the national toxicovigilance network about any adverse effects involving spirulina that had been brought to their attention. The results of this enquiry were submitted in the form of a report, which has been summarised in Section 3.2.1.

ANSES contacted the health agencies in various European countries, Canada and the United States to obtain any insights they may have gained from surveillance and expertise on the safety of food supplements containing spirulina. The responses provided have been summarised in Section 3.2.2.

Lastly, the Federation of French Spirulina Producers (FSF) was consulted by ANSES in the framework of this internal request. The Federation was invited to respond to questions posed by ANSES and to bring to its attention any information considered useful in the framework of the assessment of the risks associated with the consumption of food supplements containing spirulina. The minutes from this hearing are provided in Annex 2.

3. ANALYSIS AND CONCLUSIONS OF THE CES AND THE WG

3.1. Spirulina in food supplements

3.1.1. Characterisation of the ingredient

Spirulina is the common name given to cyanobacteria of the genus *Arthrospira* (formerly *Spirulina).* It develops by forming blooms on the surface of bodies of water, by photoautotrophy (i.e. by converting sunlight with the help of its pigments). It grows naturally in fresh water that is warm (25°C), alkaline (pH 8-11.5), and rich in carbonates and bicarbonates, but also in nitrates, phosphates and iron, in lakes or temporary pools. This cyanobacterium is organised into a succession of cells forming coiled filaments (Komárek *et al.* 2014, Turpin 1827).

The genus *Arthrospira* is naturally present in the intertropical regions: the alkaline lakes in Africa (Chad, Ethiopia, Tunisia), in Latin America (Mexico, Peru) and southern Asia (India, Sri Lanka, Thailand). The species *Arthrospira fusiformis* (syn. *Spirulina platensis*) is the most widespread, with a broad distribution (Africa, Asia, South America), whereas *Arthrospira maxima* (syn. *Spirulina maxima*) is confined to Central America (Mexico, California).

Spirulina is used for food purposes, mainly in the form of supplements, due to its nutritious potential, or as a colouring. It is also used in animal feed.

3.1.1.1. Classification

The genus *Arthrospira* has the characteristics of both a bacterium (absence of a nucleus and presence of a Gram-negative cell wall) and a eukaryotic microalga (presence of chlorophyll and photosynthesis capability *via* phycobiliproteins). It is currently regarded as a cyanobacterium, therefore belonging to the domain of the Eubacteria.

Spirulina's taxonomy has often been revised. The genus name *Spirulina* is no longer used and the term "spirulina" encompasses several species of similar morphology and composition, belonging to the genus *Arthrospira* in the order Oscillatoriales, family Microcoleaceae (Sili, Torzillo, and Vonshak 2012, Komárek *et al.* 2014).

The commercial strains belong to the following species:

- *Arthrospira maxima* Setchell & N.L.Gardner (syn. *Spirulina maxima* (Setchell & N.L.Gardner) Geitler);
- *Arthrospira fusiformis* (Voronikhin) Komarek & J.W.G.Lund (syn. *Arthrospira platensis* (Nordstedt) Gomont, *Spirulina fusiformis* Voronikhin);
- *Arthrospira indica* Desikachary & N.Jeeji Bai (syn. *Spirulina fusiformis sensu* Jeeji Bai & Seshadra, *Arthrospira platensis* f*. granulata* Desikachary).

In this Opinion, in the absence of precision in the literature data or for practical reasons, the term "spirulina" will be used.

3.1.1.2. Regulatory status

Spirulina is regarded as a foodstuff subject to the rules relating to such products in the European Union. The species *A. maxima* and *A. platensis* are listed under the genus name *Spirulina* (Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin).

Three species of spirulina are listed in the Order of 24 June 2014 establishing the list of plants, other than fungi, authorised in food supplements and the conditions of their use (Official Journal (JORF) No 0163 of 17 July 2014). No substance to be monitored and no restriction is mentioned for spirulina (Table 1). The species *Spirulina major* Kützing ex Gomont refers to a European species (Finistère and Germany) that is not referenced in the classifications currently in force (Gomont 1893).

Table 1: Extract of the Order of 24 June 2014, concerning spirulina

Scientific NAME	FAMILY	Vernacular NAME	PARTS used	SUBSTANCES to be monitored	RESTRICTIONS
Spirulina major Kützing ex Gomont	Pseudanabaenaceae	Spirulina	all parts		
Spirulina maxima (Setchell & N.L.Gardner) Geitler	Pseudanabaenaceae	Spirulina	all parts		
Spirulina platensis (Gomont) Geitler	Pseudanabaenaceae	Spirulina	all parts		

The list of plants eligible for Article 15 of Decree 2006-352 of 20 March 2006 concerning food supplements also lists "*Spirulina* P.J.F.Turpin ex M.Gomont". The four "spirulina" entries on this list are not subject to any restrictions (Table 2).

Table 2: Extract from the list of plants eligible for Article 15 of Decree 2006-352[1]

SCIENTIFIC NAME	RESTRICTIONS ON USE & RECOMMENDATIONS FOR SUBSTANCES TO BE MONITORED	PART THAT CAN BE USED
Spirulina major Kützing ex Gomont		Whole plant
Spirulina maxima (Setchell & N.L.Gardner) Geitler		Whole plant
Spirulina platensis (Gomont) Geitler		Whole plant
Spirulina P.F.J.Turpin ex M.Gomont		Whole plant

Spirulina has not undergone any risk assessment by the European Food Safety Authority (EFSA). It is not regarded as medicinal in France or Europe (there are no data from the French Health Products Safety Agency – ANSM, no registration on the French Pharmacopoeia, no data from the European Medicines Agency – EMA).

Spirulina is a foodstuff in France and is recognised as a food by the Food and Agriculture Organization of the United Nations (FAO) (Piccolo 2011).

The *Codex Alimentarius* lists spirulina extract as a food additive (colour). Spirulina extracts are also food additives approved for use as food colourings in the United States, China, Japan and Korea. Spirulina extracts or concentrates are covered by specifications and maximum concentration limits in food (around 0.5 to 2% m/m), which are listed in Annex 1 of the 2016 *Codex Alimentarius*. The chemical composition of such extracts is not described.

The US Food and Drug Administration (FDA) has assigned the status Generally Recognised As Safe (GRAS) to spirulina, at doses of 3 to 6 g/d (FDA 2003). Following an assessment of this ingredient by the "*United States Pharmacopoeia Safety Evaluation*" working group (Marles *et al.* 2011), a monograph is available in the *USP Dietary Supplements Compendium* for *Arthrospira platensis* (USP 2015).

3.1.1.3. Production of spirulina

Spirulina is an ancient traditional food, mainly known for its dietary uses in South America and in Chad (dried on straw, a form known as *dihé*). In this framework, it is harvested from the wild or cultivated in a traditional way. From the 1980s, industrial production of spirulina for food purposes emerged in Mexico, Taiwan, the United States, Thailand, Japan, Israel, etc. (Ciferri and Tiboni 1985, Vonshak *et al.* 1983, Vonshak and Richmond 1988). This developed in parallel with the production of certain microalgae (*Chlorella*, *Dunaliela*, *Aphanizomenon* spp.), using the same types of facilities. Production in reactors fitted with lighting systems (photobioreactors) enabling

[1] Accessed on 13 June 2017.

autotrophic development of spirulina emerged at the beginning of the 2000s (Muller-Feuga, Pulz, and Brault 2004).

Worldwide production is high. For example, the FAO noted the following tonnages in 2010, for the major producers: 62,300 tonnes in China and 6000 tonnes in Chile (FAO 2010). The Federation of French Spirulina Producers reported French production of the order of 30 tonnes/year (Annex 2).

3.1.1.4. Methods of consumption of spirulina

Traditional food consumption of spirulina is well documented in certain countries such as Chad and Mexico. Intakes can reach or exceed 50 g per week (dry mass) in traditional consumption areas (Ciferri and Tiboni 1985, Delpeuch, Joseph, and Cavelier 1976). A study conducted in Chad reported consumption of spirulina in the form of a sauce accompanying millet at 1 to 6 out of the 10 daily food intakes, at a rate of 9 to 13 g per meal (dry mass) (Delpeuch, Joseph, and Cavelier 1976). In supplementation studies focusing on treatment of malnutrition, the doses received were generally 5 to 10 g/d (Gershwin and Belay 2008, Ngo-Matip *et al.* 2015, Simpore *et al.* 2006, Winter *et al.* 2014, Yamani *et al.* 2009).

In France, spirulina is marketed as a bulk foodstuff, in the form of a powder or food supplement, as capsules or tablets that can provide around 0.25 to 5 g of spirulina per day (Cornillier, Korsia-Meffre, and Senart 2008).

3.1.1.5. Composition

The composition of spirulina has been described in the scientific literature (Ciferri and Tiboni 1985, Gershwin and Belay 2008, Holman and Malau-Aduli 2013, Santillan 1982, Falquet and Hurni 2006). The authors do not differentiate between the species *A. maxima* and *A. platensis*. Spirulina contains the following nutrients, with content expressed relative to the dry matter:

- **Carbohydrates**

The carbohydrate content of spirulina varies from 14 to 19% (Gershwin and Belay 2008, Holman and Malau-Aduli 2013). A study reported that carbohydrates constitute 15% of the dry matter, of which 12% are polysaccharides, represented by 2% glucosans and 10% rhamnosans (Quillet 1975). The other carbohydrates described are mono- or disaccharides (glucose, fructose, sucrose) and phosphated cyclitols. Traces of glycogen have also been described. The presence of inositol (350 to 850 mg/kg) and meso-inositol phosphate has been mentioned (Santillan 1982, Falquet and Hurni 2006).

- **Proteins**

The protein content ranges from 60 to 70% of the dry matter of spirulina, with a high proportion of essential amino acids (47%), and sulphur amino acids being slightly under-represented (Gershwin and Belay 2008, Holman and Malau-Aduli 2013). In particular, spirulina contains phenylalanine, for which Gershwin and Belay (2008) reported a mean concentration of 2.75 g/100 g of dry powder.

Spirulina contains phycobiliproteins, mainly C-phycocyanin. The levels reported in the literature vary (5 to 15%) (Boussiba and Richmond 1979, Holman and Malau-Aduli 2013, Niu *et al.* 2007, Sarada, Pillai, and Ravishankar 1999). In a study that reported the presence of around 14% C-phycocyanin, a level of 4.7% phycocyanobilin was measured (McCarty 2007).

The monograph devoted to *Arthrospira platensis* in the *USP Dietary Supplements Compendium* selected a minimum level of 52% for protein and 5% for C-phycocyanin in particular (USP 2015).

- **Fats**

The total fat content of spirulina (mainly in the form of di- and triglycerides) is generally less than 10%. The fatty acid composition of spirulina is highly variable. Palmitic (25-60%), γ-linolenic (4-40%), linoleic (5-30%) and oleic (5-17.5 %) acids are predominant (Diraman, Koru, and Dibeklioglu 2009, Holman and Malau-Aduli 2013, Hudson and Karis 1974, Habib *et al.* 2008). The presence of long-chain polyunsaturated fatty acids is inconsistent (docosahexaenoic and eicosapentaenoic acid; typically < 5%) (Diraman, Koru, and Dibeklioglu 2009). The literature reports the minority presence of sulpholipids (esters of fatty acids, glycerol and sulphated sugars) (Kwei *et al.* 2011). The unsaponifiable lipid fraction (around 13% of the total lipid fraction) contains sterols, pentacyclic triterpenes, hydrocarbons and pigments (Falquet and Hurni 2006).

The monograph of the *USP Dietary Supplements Compendium* for *Arthrospira platensis* advocates determining the chromatographic profile of fatty acids using HPLC-UV (USP 2015).

- **Vitamins**

  - Water-soluble vitamins

Spirulina contains vitamins from group B (Santillan 1982, Clement, Giddey, and Menzi 1967): B1 (around 40-50 mg/kg), B2 (30-45 mg/kg), B3 (130-150 mg/kg), B5 (4.5-25 mg/kg), B6 (1-8 mg/kg) and B12 (0.1-2 mg/kg) (Gershwin and Belay 2008, Holman and Malau-Aduli 2013). The vitamin B12 in spirulina is made up of at least two analogues, of which the major (80%) is pseudo-vitamin B12, which does not bind to the intrinsic factor and is therefore inactive (Watanabe *et al.* 1999, Watanabe 2007, Herbert and Drivas 1982). Spirulina supplementation of children deficient in vitamin B12 has proved to be ineffective for correcting macrocytic anaemia (Dagnelie, Van Staveren, and Van den Berg 1991).

The American Academy of Nutrition and Dietetics does not consider spirulina to be a reliable source of vitamin B12 for vegetarian and vegan populations (Melina, Craig, and Levin 2016).

  - Fat-soluble vitamins

    - Carotenoids

Among the carotenoids present, β-carotene (provitamin A) is in the majority (50 to 80%). Concentrations of β-carotene measured in spirulina are of the order of 1.4 to 1.7 mg/g (Gershwin and Belay 2008, Holman and Malau-Aduli 2013). Consumption of 5 g/day of spirulina (maximum quantity recommended by some food supplements) therefore provides from 7 to 8.5 mg of beta-carotene. The maximum recommended daily intake of beta-carotene via food supplements has, however, been estimated at 7 mg/d (AFSSA 2009).
Only a population reference intake (PRI) for vitamin A, in retinol equivalent, has been established. In their study, Wang *et al.* (2008) reported that when consumed with 22 g of fat, 4.5 mg of beta-carotene provided by spirulina enables an intake of 1 mg of vitamin A. Thus, the quantities of beta-carotene contained in 5 g of spirulina, expressed in retinol equivalent (1600 to 1900 µg RE), may exceed the PRIs, which are 750 µg RE/d in men and 650 µg RE/d in women (ANSES 2016), without by themselves exceeding the upper intake level set at 3 mg/d for vitamin A for considerations of hepatotoxic effects and teratogenic effects (EFSA 2015).

The other carotenoids are provitamin A (β-cryptoxanthin) or non-provitamin A (zeaxanthin, myxoxanthophyll, canthaxanthin, diatoxanthin and oscillaxanthin and echinenone) (Palla and Busson 1969, Marles *et al.* 2011, Habib *et al.* 2008, Wang *et al.* 2008). The levels described are around 0.4% in total carotenoids (Santillan 1982, Clement, Giddey, and Menzi 1967).

The monograph devoted to *Arthrospira platensis* in the *USP Dietary Supplements Compendium* selected minimum levels of 0.12% β-carotene and 0.38% total carotenoids (USP 2015).

- Vitamin E

Spirulina has a vitamin E content of 100-190 mg/kg (Holman and Malau-Aduli 2013, Koru 2012, Santillan 1982).

➢ **Minerals**

The mineral content of spirulina varies greatly depending on the production method and the harvesting area for wild spirulina. Spirulina contains potassium, calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, zinc and fluorine (Diraman, Koru, and Dibeklioglu 2009, Holman and Malau-Aduli 2013, Ötleş and Pire 2001, Santillan 1982, Vicat, Doumnang Mbaigane, and Bellion 2014, Boudène, Collas, and Jenkins 1975).

➢ **Other**

- Chlorophyll a

Chlorophyll a is the only chlorophyll found in cyanobacteria. The phycobiliprotein/chlorophyll a ratio varies from 2.5 to 4.5 depending on the salinity of the medium and the culture conditions (Leema *et al.* 2010). Chlorophyll a levels are around 1.1-1.5% (Koru 2012). The monograph devoted to *Arthrospira platensis* in the *USP Dietary Supplements Compendium* selected a minimum level of chlorophyll of 0.7% (USP 2015).

- Nucleic acids

The nucleic acid (DNA and RNA) composition of *S. platensis* and *S. maxima* has been reported, with concentrations of 4.29 and 4.25 g/100 g of dry matter respectively (of which 3.56 g and 3.66 g of RNA) (Jassey, Berlot, and Baron 1971), which can represent a non-negligible intake of purine bases.

3.1.2. Risk of contamination

Spirulina is liable to contain various contaminants. Firstly, cyanobacteria from other genera and their toxins have been identified in batches of spirulina. Secondly, the presence of trace metal elements has been reported in wild or cultivated spirulina. The presence of other bacterial species is also possible.

3.1.2.1. Contamination by cyanobacteria and associated toxins

➢ **Cyanotoxins**

Cyanotoxins are produced by several genera of cyanobacteria. It should be noted that the production of cyanotoxins by the genus *Arthrospira* has not been described in the literature, with the exception of the work by Ballot *et al.*, which reported the presence of microcystins and anatoxin-a in two samples of *A. fusiformis* isolated from certain lakes in Kenya (Ballot *et al.* 2004, Ballot *et al.* 2005, Krienitz *et al.* 2003).

Genes for the biosynthesis of cyanotoxins have been identified in batches of spirulina, reflecting contamination (Heussner *et al.* 2012, Marles *et al.* 2011).

Cyanotoxins can be classified into three categories according to their toxicity: hepatotoxins, neurotoxins and dermatoxins (AFSSA and AFSSET 2006, Bernard 2014).

- Hepatotoxins: microcystins

Microcystins are cyclic heptapeptides essentially produced by the genera *Microcystis*, *Aphanizomenon*, *Planktothrix* and *Dolichospermum* (ex. *Anabaena*). Around a hundred molecules have been described. They cause hepatocyte lysis, general digestive symptoms and liver failure in acute exposure, as well as hepatocarcinomas in chronic exposure. They are also responsible for kidney failure and neurological disorders. The WHO has defined a tolerable daily intake (TDI) of 0.04 µg/kg/d for chronic exposure (WHO 2003).

The methods used to screen for microcystins can be high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), immunofluorescence using antibodies targeting the microcystins (ELISA), or enzyme inhibition measurement (protein phosphatase 1). Some authors have reported similar results for these three methods (Heussner *et al.* 2012, Lawrence *et al.* 2001); these results cannot however be generalised because of the specificity of the methods, with different principles of detection, and matrix effects. Assessing the cytotoxicity of extracts on certain microorganisms (*Vibrio fischeri*) and screening by PCR of genes encoding enzymes for biosynthesis of microcystins (mcyE gene) have also been used in some studies.

While most of the studies have concluded as to an absence of microcystins in spirulina (Rzymski *et al.* 2015, Heussner *et al.* 2012, Manali *et al.* 2016, Vichi *et al.* 2012), these toxins have nevertheless been detected in spirulina batches at levels of generally around 0.1 µg/g (for the batches presented as containing spirulina only; Table 3). In comparison, food supplements containing the cyanobacterium *Aphanizomenon flos-aquae* (known as Klamath blue-green alga) show higher levels of contamination, which are cause for concern (up to 18 µg/g) (Vichi *et al.* 2012, Gilroy *et al.* 2000, Heussner *et al.* 2012).

The monograph devoted to *Arthrospira platensis* in the USP *Dietary Supplements Compendium* adopted a maximum level of microcystins of 0.5 mg/kg by ELISA (USP 2015). In contrast, the Order of 24 June 2014 does not specify that microcystins are substances to be monitored in food supplements containing spirulina. It does however state this for products containing Klamath alga (*Aphanizomenon flos-aquae*), which must contain less than 1 µg/g of microcystins. However, this limit has not been scientifically substantiated. Given the tolerable daily intake established by the WHO, this limit should be reassessed. However, the identification and assaying methods are not detailed and the manufacturer has an obligation to use suitable methods.

- Neurotoxins

Anatoxins, with an alkaloid structure, are neurotoxins produced by many genera of cyanobacteria: *Dolichospermum* (ex. *Anabaena*), *Aphanizomenon* and *Phormidium*, but also *Microcystis*, *Planktothrix* and *Raphidiopsis*. They can be fatal (paralysis of striated skeletal muscles including the respiratory muscles), in particular at a high dose (LD_{50} >5000 µg/kg by the oral route in mice) (AFSSA and AFSSET 2006).

They are represented by anatoxin-a and homoanatoxin-a, which induce depolarisation of the post-synaptic membrane and inhibit acetylcholinesterase. The anatoxins degrade into epoxy- and dihydro-anatoxin-a and homoanatoxin-a, which are non-toxic.

Anatoxin-a and derivatives of this molecule have been detected, in low concentrations, in batches of spirulina (Table 3). In a study carried out on samples of spirulina marketed in Italy, the presence of anatoxins was not detected; in contrast, non-toxic anatoxin degradation products were found in three of the five samples analysed by LC-MS/MS: dihydrohomoanatoxin-a (9 to 10 µg/g) and an isomer of epoxyanatoxin-a (18 and 19 µg/g) (Draisci *et al.* 2001). The other studies concluded as

to an absence of anatoxins (Rawn *et al.* 2007, Rzymski *et al.* 2015), or to an extremely low content, below the limits of quantification (10 ng/g) (Rellán *et al.* 2009).

Saxitoxins and BMAA (β-*N*-methylamino-L-alanine), two other neurotoxins, have not been detected in marketed batches of spirulina or in naturally occurring spirulina (Dittmann and Wiegand 2006, Marles *et al.* 2011, McCarron *et al.* 2014, Svrcek and Smith 2004).

These three neurotoxins are nevertheless known to be difficult to analyse and quantify.

- Dermatoxins

These alkaloids are produced by strains of cyanobacteria developing in the marine environment (lyngbyatoxins, aplysiatoxin, debromoaplysiatoxin) (Bernard 2014). They have not been screened for in batches of spirulina.

Table 3: Screening for cyanotoxins in food supplements and foods containing spirulina

Food supplements (FSs) and foods tested	Cyanotoxins detected (concentrations expressed in dry weight)	Reference
Microcystins		
17 FSs: 9 intended for human food, 8 intended for animal feed (India, Japan, Thailand, Germany).	Microcystins detected (multiplex HRM qPCR) only in products intended for animal feed (no level reported).	Manali *et al.* (2016)
26 FSs including 6 containing spirulina and 11 made of *A. flos-aquae* (Italy).	Microcystins not detected in the FSs containing spirulina but detected in the FSs containing *A. flos-aquae* (up to 5.2 µg/g) using LC-MS/MS. PCR showed that there was contamination by *Microcystis* in positive samples.	Vichi *et al.* (2012)
18 FSs including 5 containing spirulina (Germany).	Microcystins detected in a single FS containing spirulina (0.1 µg/g by PPIA; 0.2 µg/G by Adda-ELISA and <LD by LC-MS/MS), but in all the FSs containing *A. flos-aquae*. McyE gene present in almost all the FSs producing microcystins.	Heussner *et al.* (2012)
36 FSs containing spirulina (China).	Microcystins detected (LC-MS/MS) in 94% of the FSs containing spirulina (ND)-163 ng/g)	Jiang *et al.* (2008)
19 FSs including 10 containing spirulina and 9 made with *A. flos-aquae* (Switzerland)	Microcystins not detected in the FSs containing spirulina but detected in 6 of the FSs containing *A. flos-aquae* (up to 4 µg/g) by UPLC-TOF.	Ortelli *et al.* (2008)
40 FSs containing unspecified cyanobacteria (Italy).	Microcystins detected by two methods: ND-3.3 µg/g (ELISA) *vs* 0.3-4.6 µg/g (LC-MS/MS) 0.10-1.30 µg/g (ELISA) vs ND-1.42 µg/g (LC-MS/MS)	Bruno *et al.* (2006)

Food supplements (FSs) and foods tested	Cyanotoxins detected (concentrations expressed in dry weight)	Reference
11 FSs including 3 containing spirulina, 6 made of *Chlorella* and 2 containing an unidentified "blue-green" alga (Taiwan)	Microcystins detected (ELISA) in the 3 FSs containing spirulina (28-78 ng/g), in 3 FSs containing *Chlorella* (<20-36 ng/g) and in the FSs made of "blue-green" alga (48-51 ng/g)	Yu *et al.* (2002)
15 FSs containing spirulina (USA)	Microcystins detected (ELISA): 0.15 and 0.52 µg/g in average concentrations (min <LD and max=2.12 µg/g)	Gilroy *et al.* (2000)
Anatoxins		
5 commercially available FSs (tablets, capsules) (Italy).	Dihydrohomoanatoxin-a (n=3/5): 9-10 µg/g Epoxyanatoxin-a (n=2/5): 18-19 µg/g	Draisci *et al.* (2001)
90 commercially available FSs also containing other algae (Australia, Portugal).	Traces detected by LC-UV but not by LC-MS/MS. The authors concluded as to an absence of contamination.	Rawn *et al.* (2007)
39 products: 2 animal feeds and 37 FSs, including 34 containing spirulina (different geographical origins).	Anatoxin-a detected: - In the 2 batches of animal feed (fish, birds): 2.5 and 33 µg/g. - In 3 FSs containing spirulina, at levels below the limit of quantification.	Rellán *et al.* (2009)

ND: Not detected
PPIA: Phosphatase inhibition assay

- **Cyanotoxin-producing cyanobacteria**

Cyanotoxin-producing cyanobacteria belong to several genera. Their development in certain freshwater lakes has led to cases of animal and human poisoning (Bernard 2014).

- Cyanobacteria identified in natural spirulina development environments

Several publications have reported the presence of toxic cyanobacteria in alkaline lakes. The large-scale presence of several toxic cyanobacteria (*Anabaena* sp., *Anabaenopsis* sp., *Phormidium* sp., *Oscillatoria* sp., *Synechococcus* sp.), associated with the presence of cyanotoxins (anatoxin-a, microcystins) has been identified in two Kenyan lakes (Krienitz *et al.* 2003).

- Cyanobacteria identified in culture conditions

Spirulina culture conditions cannot rule out the possibility of growth of other cyanobacteria that may develop in the same conditions (pH > 9.5). There is a risk of the presence of cyanobacteria other than *Arthrospira*, in particular of the genera *Anabaena*, *Anabaenopsis, Microcystis, Oscillatoria* or *Phormidium* (Jourdan 2011).

- Cyanobacteria identified in commercial samples

The aforementioned analyses by Draisci *et al.* (2001) revealed contamination by an anatoxin-producing cyanobacterium in samples of spirulina marketed in Italy.

- **Means of controlling contamination by cyanobacteria or cyanotoxins**

The presence of other cyanobacteria in the culture medium may be related to contamination from the water used or to a poor-quality inoculum.

The strains used for the inoculum may be wild (from lakes in the vicinity) or may be commercial strains of varying purity. The quality of the inoculum determines contamination by other, potentially toxic, micro-organisms. Good practices regarding sampling and isolation of the strain used for inoculating the culture ponds and controlling the culture conditions can limit the risk of the presence of other cyanobacteria. For example, production control methods ensuring a high bicarbonate concentration help limit the development of *Chlorella* (Vonshak *et al.* 1983, Vonshak and Richmond 1988).

A microscopic analysis is recommended for *Arthrospira platensis* in the monograph of the *USP Dietary Supplements Compendium* (USP 2015).

A French-language guide to traditional production of spirulina, used and cited by the producers, proposes a grid for the microscopic identification of toxic cyanobacteria to be monitored (Jourdan 2011).

The Federation of French Spirulina Producers also offers its members a guide to good hygiene practices, including the monitoring of strains used in production and regular controls of the spirulina produced, in particular by screening for microcystins.

Nevertheless, in the case of traditional production in infrastructures that are unequipped for microbiological testing, or with staff that are untrained in quality control management of the spirulina produced, contamination remains possible.

Most products containing exclusively spirulina (*Arthrospira* spp.) that have undergone contamination studies do not have a worrying level of cyanotoxins. The data available in the literature cannot be used to reach any conclusion as to the microcystin production potential of *Arthrospira* spp. Moreover, contamination by other cyanotoxin-producing cyanobacteria is possible in traditional or industrial settings.

Consequently:

- the absence of contamination by other cyanobacteria should be systematically verified when selecting the inoculum and during the various stages of production;

- concerning microcystins, the WHO has established a TDI of 0.04 µg/kg/d. A threshold should be established for microcystins in food supplements containing spirulina that takes into account other dietary intakes of microcystins.

3.1.2.2. Contamination by other bacteria (excluding cyanobacteria)

The strongly alkaline conditions of spirulina production (pH of around 10) limit the development of most pathogenic micro-organisms (*Listeria*, *Salmonella*, coliforms, etc.). Nevertheless, contamination can occur during subsequent handling (i.e., during harvesting, washing, drying, storage, or even packaging, etc.), mainly depending on the hygiene practices and the water used.

A study examined the bacteria associated with spirulina from different origins: spirulina dried on straw (dihé) from Lake Chad, samples from Lake Texcoco (Mexico), from an Algerian pond and an experimental French production site at Rueil-Malmaison (Jacquet 1975). The micro-organisms screened for were as follows: aerobic mesophilic flora, anaerobic flora, yeasts and moulds, coliforms, faecal streptococci, staphylococci, sulphite-reducing anaerobic bacteria and amoebae. The sample from Chad had the highest contamination, predominantly by bacilli and a few faecal streptococci. The presence of other germs was also reported: group D streptococci, enterobacteria

(*Enterobacter, Proteus, Citrobacter*). Yeasts and moulds were very rare (*Rhodotorula, Cladosporium, Aspergillus, Penicillium*). Diatoms were detected (*Navicula, Aristerionella*) as well as protists of the genera *Stylonychia* and *Spiromonas*. Contamination came from the natural environment or was introduced during handling (enterobacteria).

Bacteria of the *Clostridium* genus have been identified in food supplements containing spirulina sold in Europe: the three batches tested, from the same manufacturer, exceeded the standards accepted in food, two with levels greater than 10^7 CFU/g (Hoekstra *et al.* 2011).

A study conducted on 31 commercial samples of spirulina sold on the Greek market revealed bacterial contamination (Vardaka *et al.* 2016): 469 heterotrophic bacterial species were found, some of which may be pathogenic among the genera identified: *Pseudomonas*, *Flavobacterium*, *Vibrio*, *Aeromonas*, *Clostridium*, *Bacillus*, *Fusobacterium*, *Enterococcus*. The viability of these species was not evaluated. The production sites of the samples were very varied.

Bacterial contamination of spirulina can occur during handling (harvesting, washing, drying, storage, packaging, etc.). Nevertheless, contamination at concentrations exceeding the usual food standards seems to be rare for finished products. Moreover, the presence of bacterial contaminants in batches of commercial spirulina does not seem to have been formally associated with adverse effects.

3.1.2.3. Contamination by trace metal elements

The phycobiliproteins in spirulina have strong chelating properties (Chen and Pan 2005) and the trace metal element (TME) content of spirulina is directly correlated with the quality of the water. The presence of TMEs has been reported in wild or commercial spirulina.

- **Wild spirulina**

A high arsenic (As) content in wild samples collected in Chad has been shown, as well as a high lead (Pb) content in a sample grown in Burkina Faso. This pollution may be related to the harvesting method, which incorporates mud from the *wadis* with the biomass of spirulina (Vicat, Doumnang Mbaigane, and Bellion 2014). In addition, high levels of lead have been detected in Cuban commercial samples. This may be linked to contamination of the production site (Campanella, Crescentini, and Avino 1999).

- **Commercial spirulina (food and food supplements)**

Many studies have been devoted to the TME content of commercial spirulina (chromium, cadmium, arsenic, lead, mercury, nickel). The accessible studies are summarised in Table 4.

Regulation (EC) No 629/2008 established the following maximum limits for food supplements in Europe: lead (3 mg/kg), cadmium (1 mg/kg for food supplements in general and 3 mg/kg for food supplements containing seaweed) and mercury (0.1 mg/kg). In addition, the proposed monograph in the USP *Dietary Supplements Compendium* for *Arthrospira platensis* provides specifications for lead (≤ 1 mg/kg), cadmium (≤ 0.5 mg/kg), mercury (≤ 0.5 mg/kg) and arsenic (≤ 1 mg/kg) (USP 2015).

Some of the studies presented in Table 4 show levels in one or more of these TMEs that are higher than these limits.

Table 4: Detection of trace metal elements in food supplements and foods containing spirulina

Products, origin	TME concentrations expressed in mg/kg of dry weight	Reference
2 food supplements	**Total As: 3.2 and 3.3** Cd, Cr, Hg, Ni, Pb and Zn: quantified but values not detailed	Rzymski *et al.* (2015)
25 food supplements	Hg: 0.002-0.028	Al-Dhabi (2013)
25 food supplements	Cr: 0.003-0.018 Cd: 0.003-0.069 As: 0.006-0.578 Pb: 0.100-1.206	Al-Harbi (2012)
5 batches of commercial spirulina (Cuba, Italy, Mexico, USA)	Cd: not detected Cr: 9.02-26 Hg: 0.069-0.096 Ni: 0.08-0.1 **Pb: 6.95-12.1**	Campanella, Crescentini, and Avino (1999)
3 batches of commercial spirulina from the same brand (USA)	**Cd**: 0.2-0.6 Ni: 1.6-2.7 **Hg: 9.8-17.2** **Pb:** 2.5-**4.4**	Johnson and Shubert (1986)
8 batches of commercial spirulina (Thailand, Mexico, USA, Taiwan, Israel)	Cd: 0.3-0.7 Cr: 2.2-6.5 Ni: 4.2-15.0 (2 samples > 10) **Hg: 9.1-24.4** **Pb:** 1.3-**6.7**	Sandau, Sandau, and Pulz (1996)
Commercial spirulina (India)	As: 0.97 **Pb: 3.95** Hg: 0.07 Cd: 0.62	See for review Gershwin and Belay (2008)
1 batch of commercial spirulina (Mexico)	**As: 2.9** **Pb: 5.1** **Hg: 0.5** Cd: 0.5	See for review Gershwin and Belay (2008)
Commercial spirulina (Chad)	**As: 1.8** **Pb: 3.7** **Hg: 0.5** Cd: not detected	See for review Gershwin and Belay (2008)
1 batch of spirulina cultivated experimentally for commercial purposes (Saudi Arabia)	As: 0.002 Pb: 0.109 Hg: 0.008 Cd: 0.031	Al-Homaidan (2006)
Commercial spirulina (USA)	As: < 1.0 Pb: < 1.0 Hg: < 0.05 Cd: < 0.05	Al-Homaidan (2006)

Products, origin	TME concentrations expressed in mg/kg of dry weight	Reference
Commercial spirulina (India)	**As: 1.1** Pb: 2.5 Hg: 0.1 Cd: 1.0	Al-Homaidan (2006)

As: arsenic, Cd: cadmium, Cr: chromium, Hg: mercury, Ni: nickel, Pb: lead.
In bold: values above the maximum tolerable limits defined in Regulation (EC) No 629/2008 (Pb, Cd, Hg), or in the Monograph of the *USP Dietary Supplements Compendium* (As).

While the majority of studies seem to show tolerable TME concentrations, certain analyses show excessive levels of one or more TMEs (arsenic, lead, mercury) compared to the maximum levels in force in the European Union or the specifications in the *USP Dietary Supplements Compendium.* A risk of exposure to concentrations higher than the international recommendations is therefore possible, especially for spirulina productions with insufficient controls.

3.2. Adverse effects

3.2.1. Cases from nutrivigilance

Between the establishment of the nutrivigilance scheme and the month of February 2017, ANSES received 49 reports of adverse effects likely to be associated with the consumption of food supplements containing spirulina. Ten of these were analysed for their causality (Table 5), while the others were not sufficiently documented to be analysed (due for example to the dates of consumption being unknown, or the product not being clearly identified). To help identify the role of these food supplements in the reports received, ANSES analysed the causality of 10 sufficiently documented cases, with the help of the Working Group on "Nutrivigilance", using the method defined in ANSES's Opinion of 11 May 2011 (ANSES 2011).

Case 2011-112 was the subject of a publication in 2014 because of the great severity of the adverse effect (allergic facial angioedema) and the very likely causality (ANSES 2014).

Case 2013-203 concerned a 48-year-old woman without any particular prior history, who had taken three tablets of a food supplement containing spirulina. Four hours after consumption, stomach cramps, nausea, vomiting and liquid stool occurred. The consumer's condition improved in 3 to 4 hours without any symptomatic treatment. On two subsequent occasions, the consumer again took tablets of the same food supplement; the same clinical picture reappeared each time. The Working Group on "Nutrivigilance" recognised the food supplement's role in the onset of digestive disorders, with very likely causality.

Case 2013-107 involved several products falling within the nutrivigilance scheme. It concerned a 35-year-old man who for six months had consumed energy drinks intended for athletes during sport training sessions (carried out 4 to 6 times a week) and after intensive sessions (not more than twice a week). On 15 April 2013, he began consumption of a food supplement containing spirulina, with doses increasing from one and then two tablets a day, up to ten tablets a day. On 8 May 2013, violent muscle pains in the thighs appeared. Two days later, a vasovagal syncope led the consumer to visit the hospital emergency department, and he ceased taking the food supplement. On arrival at the emergency department, his walking range was limited to 300 metres due to the pain, which was not relieved by level 1 and 2 analgesics. His creatine kinase (CPK)

levels were 68,000 IU/L. Most of the examinations performed were negative (infectious test, viral serologies, autoimmune test, EMG) except for the muscle MRI, which found inflammatory lesions (myositis). Muscle recovery was observed. Nevertheless, a thrombosis of one of the two peroneal veins occurred on 24 May 2013, requiring prescription of an anticoagulant treatment. The Working Group on "Nutrivigilance" recognised the food supplement's role in the onset of muscle disorders, with likely causality. This adverse effect may have occurred through a mechanism involving the pro-oxidant nature at high doses of beta-carotene provided by these spirulina doses in a situation of hyperoxia and ischaemia-reperfusion, as induced by the physical exercise (Rutkowski and Grzegorczyk 2012).

Case 2016-030 concerned a 51-year-old woman, without any particular prior history, who had consumed two food supplements, the first from 15 September 2015 and then the one containing spirulina from 1 October 2015, in a context of hair loss. On 16 November 2015, during a systematic examination performed subsequent to a face rash (which predated the consumption of food supplements), kidney failure was diagnosed with a serum creatinine level at 135.3 µmol/L, combined with a discrete polyuria-polydipsia. It should be noted that the previous serum creatinine level had been normal, at 81.4 µmol/L, on 1 October 2013. Additional examinations found no proteinuria, or micro-albuminuria, or anomaly of the complement system. The ultrasound of the urinary tract was normal. On 21 November 2015, the subject ceased taking the food supplements and an improvement was observed over the following 15 days (serum creatinine at 80 µmol/L). The Working Group on "Nutrivigilance" recognised the spirulina food supplement's role in the onset of the serum creatinine anomalies, with likely causality.

In the other sufficiently documented cases reported to the nutrivigilance scheme, the Working Group concluded that causality was low regarding the food supplement's role in the onset of the observed disorders (three possible cases, two doubtful cases and one case excluded).

Ten of the reports received in nutrivigilance were sufficiently documented to be analysed. These reports included four with high causality (likely or very likely) with heterogeneous effects reported (allergic, digestive, rheumatic, uro-nephrological). Other types of effects (dermatological, liver, endocrine or metabolic) have also been reported but the causalities established for these cases are low.

Table 5: Sufficiently documented cases received by the nutrivigilance scheme between 2009 and February 2017

Registration no.	Species used	Sex and age of the consumer	Adverse effect(s)	Type(s) of adverse effect(s)	Severity of the clinical picture	Chronological score[1]	Semiological score[2]	Intrinsic causality[3]
2011-112	*Spirulina platensis*	M, 35 years	angioedema	allergy	level 3	C3 (timeframe consistent, progression suggestive)	S3 (aetiology confirmed by an allergy test)	very likely
2013-203	*Spirulina platensis*	F, 48 years	digestive disorders	digestive	level 1	C4 (timeframe consistent, progression suggestive, reintroduction positive)	S2 (another hypothetical aetiology)	very likely
2013-107*	unknown	M, 35 years	myositis	rhumatology	level 3	C3 (timeframe consistent, progression suggestive)	S2 (a few aetiologies explored and excluded)	likely
2016-030*	*Spirulina maxima*	F, 51 years	kidney failure	uro-nephrology	level 1	C3 (timeframe consistent, progression suggestive)	S1 (no aetiology sought)	likely
2012-147	*Spirulina platensis*	F, 56 years	erythema multiforme and hepatic cytolysis	dermatology and hepatology	level 2	C2 (timeframe consistent, progression inconclusive)	S1 (another possible aetiology)	possible

Registration no.	Species used	Sex and age of the consumer	Adverse effect(s)	Type(s) of adverse effect(s)	Severity of the clinical picture	Chronological score[1]	Semiological score[2]	Intrinsic causality[3]
2013-007	*Spirulina maxima*	M, 9 years	erythema	dermatology	level 1	C2 (timeframe consistent, progression cannot be interpreted)	S1 (no aetiology sought)	possible
2015-343	unknown	M, 43 years	hepatitis	hepatology	level 2	C2 (timeframe consistent, progression cannot be interpreted)	S2 (a few aetiologies explored and excluded)	possible
2012-071*	unknown	pregnant F, 34 years	congenital hypercalcaemia in the child	endocrinology	level 2	C2 (timeframe unknown, progression suggestive)	S0 (other aetiology very probable)	doubtful
2015-269	unknown	F, 40 years	hypokalaemia	metabolism	level 3	C2 (timeframe consistent, progression cannot be interpreted)	S2 (a few aetiologies explored and excluded)	doubtful
2015-124*	unknown	M, 55 years	acute generalised exanthematous pustulosis (AGEP)	dermatology	level 3	C0 (timeframe inconsistent)	-	excluded

[1]The chronological score ranges from C0 to C4.
[2]The semiological score ranges from S0 to S3.
[3]The intrinsic score ranges from I0 (excluded) to I4 (very likely).
*Case involving other products falling within the nutrivigilance scheme and for which the indicated causality relates to the food supplement containing spirulina.

Alongside the cases identified by the specific nutrivigilance scheme, 29 cases of adverse effects likely to be associated with the consumption of spirulina were recorded by the French poison control centres between 2010 and 2016. These include 18 cases that occurred after the consumption of products containing only spirulina. These concerned 10 females and 8 males (median age 31 years), of whom 13 were adults and 5 were children under the age of 9. The adverse effects reported were predominantly digestive disorders (vomiting, diarrhoea, abdominal pain), but also liver damage (cytolysis or cholestasis) and dermal symptoms (erythema). The other 11 reported cases involved other products associated with spirulina consumption. The reported adverse effects also concerned the liver and the digestive system.
In addition, three suicide attempts in particular involving spirulina were the subject of reports to the poison control centres. No deaths were observed.

3.2.2. Cases identified abroad

3.2.2.1. In Europe

In November 2016, ANSES approached its European counterparts with a view to obtaining more data on the adverse effects likely to be associated with the consumption of food supplements containing spirulina. Several countries responded that no adverse effects had been brought to their attention with this type of product (Austria, Belgium, Bulgaria, Croatia, Cyprus, Denmark, Spain, Greece, Latvia, Lithuania, Poland, United Kingdom, Slovakia, Slovenia, Sweden and Switzerland). As most of them do not have a nutrivigilance scheme, adverse effects likely to be associated with the consumption of food supplements containing spirulina are not collected in a systematic manner.

In Germany, six reports of adverse effects (abdominal pain, diarrhoea, oedema, pruritus, rash, arthralgia) likely to be associated with the consumption of food supplements containing spirulina were reported between 1998 and 2016.

In Finland, one case of vomiting and diarrhoea was reported in 2013.

In Italy, eight cases of adverse effects were collected between 2007 and 2015. The consumers involved in these cases presented with arrhythmias, headaches, paraesthesia, hepatitis, rash and hyperthyroidism.

In the Czech Republic, one case of blood in the stool was reported in 2015 following consumption of a product containing spirulina and *Chlorella*, and led the Czech National Institute of Public Health to draw consumers' attention to the potential adverse effects of these two ingredients.

3.2.2.2. In the United States and Canada

ANSES also approached the US FDA (Food and Drug Administration) and Health Canada. The FDA did not send any data. On the other hand, Health Canada sent information on two cases of adverse effects, recorded in 2010:

- a woman aged 78 years, who consumed six products concomitantly, one of which contained spirulina, and presented with abdominal pain, nausea and increased amylase. She recovered within an unknown timeframe;
- an individual (age and sex unknown) who had consumed spirulina and another product concomitantly also presented with abdominal pain and vomiting. The progression is unknown.

3.2.3. Cases reported in the literature

Several cases of adverse effects likely to be associated with the consumption of food supplements containing spirulina have been reported in the literature. They concern liver disorders, rhabdomyolysis, two cases of anaphylaxis, two cases of atopic dermatitis and one of dermatomyositis.

3.2.3.1. Rhabdomyolysis

A case of acute rhabdomyolysis was reported in Greece (Mazokopakis *et al.* 2008), in a 28-year-old man suffering from myalgia and muscle weakness following consumption of a food supplement containing only spirulina (*A. platensis* from Hawaii, Solgar brand), at a dose of 3 g/d, for one month. The consumer said he was a non-smoker, was not taking any medicinal products or other food supplements, and did not consume alcohol or drugs. His CPK was 9000 IU/L (N: 40-226 IU/L); his plasma myoglobin was 2243 µg/L (N: 0-70 µg/L); his ALT and AST transaminases were also high (180 IU/L and 288 IU/L, respectively; N: 0-45), as well as serum LDH (1265 IU/L, N: 120-450 IU/L) and aldolase (53 IU/L, N: 0-8 IU/L). After discontinuing the food supplement, the patient was given hydration and hospital supervision for 4 days. His symptoms declined and his biological parameters returned to normal. He had no predisposing factors or family history, and other causes of rhabdomyolysis were ruled out. The food supplement was not reintroduced. The potential mechanism was not elucidated. The authors suggested possible contamination by β-N-methylamino-L-alanine (BMAA), but this potentially toxic amino acid is not known to cause disorders of this type.

3.2.3.2. Hepatitis

Details of a case of hepatitis potentially associated with spirulina were published in Japan (Iwasa *et al.* 2002). A 52-year-old type-2 diabetic, hyperlipidemic, hypertensive man, being treated with simvastatin, amlodipine and acarbose for 7 months, was hospitalised for hepatitis. He had no other particular history (he had no history of infectious hepatitis and said that he did not consume alcohol or drugs, etc.). He had been taking spirulina for 5 weeks (the brand, the origin and the dose of spirulina were unknown). His AST (135 IU/L, N: 10-40 IU/L) and ALT (136 IU/L, N: 5-45 IU/L) concentrations were already high after 2 weeks of spirulina consumption, and progressed up to his admission to hospital, when they were as follows: AST 1176 IU/L, ALT 1726 IU/L, ALP 281 IU/L (N: 100-340 IU/L), γ-GT (301 IU/L, N: 50 IU/L). Cholestasis was observed, but no fibrosis or steatosis. The clinical case did not mention any dermal signs. The search for other aetiologies proved negative: no viral infection by HAV, HBV, HCV, CMV, EBV, no autoimmune disease (screening for anti-smooth muscle and anti-mitochondria antibodies). A lymphocyte stimulation test with spirulina to investigate delayed hypersensitivity proved positive. The symptoms abated on discontinuation of the medication and the food supplement. No reintroduction of the spirulina or medication was mentioned. It should be noted that simvastatin may cause hepatic disorders. Spirulina is not known to inhibit the phase I CYP3A4-mediated metabolism of this compound. In addition, an *in vitro* lymphocyte stimulation test cannot be used as evidence of the responsibility of a product (or a drug) for any adverse effects observed.

3.2.3.3. Allergy/anaphylaxis

Details of a case of anaphylaxis associated with spirulina were published in the Netherlands (Le, Knulst, and Röckmann 2014), concerning a 17-year-old man with a history of atopic dermatitis, asthma and allergic rhinitis (many known allergens, respiratory: grasses, mites, cats, dogs, and dietary: tomato, cucumber). Following ingestion of a spirulina tablet (300 mg), he developed a

facial angioedema, urticaria on the face and trunk, and nausea. This anaphylactic type reaction was treated by the emergency services. A skin prick test with the incriminated spirulina (Marcus Rohrer brand, diluted to 30 mg/mL in water) gave a positive reaction. Tests on the isolated ingredients of the tablet showed a positive reaction to *Arthrospira platensis* (0.03 mg/mL) but not to the excipients (silicon dioxide, inulin, magnesium stearate). Cross-reactions between spirulina and other allergens are unknown. Several assumptions about potential cross-reactions were mentioned; the patient had never previously consumed spirulina.

Another case of allergy to spirulina was described, in a 13-year-old child with a previous history of asthma associated with a respiratory allergy to animal dander, as well as a proven drug allergy to erythromycin and sulfadiazine-cotrimoxazole (Petrus, Assih*, et al.* 2010, Petrus, Culerrier*, et al.* 2010). On 22 January 2009, six hours after consuming five spirulina tablets (spirulina content unknown), the subject presented with skin rashes and asthma. He then received dexchlorpheniramine, methylprednisolone and nebulised terbutaline in the hospital. Faced with the persistence of a few hives under the right arm and an oedema of the upper eyelids, the patient was treated with prednisolone and ebastine. The symptoms abated. On 5 March 2009, the clinical examination and spirograph were normal. The skin prick test was positive for spirulina, as was the labial test. Three months later, the skin prick test was again positive for spirulina, as was the oral challenge test. As the child was no longer consuming spirulina, there was no recurrence of symptoms. The allergen identified was C-phycocyanin.

3.2.3.4. Atopic dermatitis

Two cases of atopic dermatitis associated with nausea, dizziness, headache and fatigue were observed in Poland. The analysis of the incriminated products (mixtures of spirulina and *Chlorella*) showed high concentrations of aluminium, cadmium, lead and mercury (Rzymski *et al.* 2015).

3.2.3.5. Dermatomyositis

One case of dermatomyositis in a 45-year-old woman with a history of fibromyalgia has been reported (Lee and Werth 2004). The incriminated product contained many ingredients (spirulina; *Aphanizomenon flos-aquae*; Cayenne chili pepper; methylsulfonylmethane). The authors concluded as to an idiosyncratic reaction with hyperproduction of TNF-α, associated with a specific genotype, potentially caused by an immunostimulant effect of spirulina. It was not possible to clearly establish causality.

3.2.4. Cases from databases

In the framework of the safety assessment of spirulina conducted by the "*United States Pharmacopoeia Safety Evaluation*" working group (Marles *et al.* 2011), reports of adverse effects associated with products containing spirulina were identified in various databases. They are summarised below:

- FDA-Medwatch: five cases of liver abnormalities, an anaphylactic reaction, an allergic reaction of another type, a Parkinsonism, a convulsive coma, a hypercalcaemia with death, and a transient ischaemia. For these cases, either there was no documentation, or the products contained multiple ingredients. The allergy case occurred in a regular consumer of spirulina who had never previously experienced problems.
- Canada Vigilance Program, between 1965 and 2009: three cases of liver disorders, a case of nephropathy and a case of skin allergy. For these cases, the causality could not be established (not documented or not admissible, or associated medication was potentially responsible).

- Cases recorded in Australia: a case of haemorrhage, a case of discolouration of breast milk, a case of phototoxicity, a case of muscle weakness and a case of discomfort with positive reintroduction. Again, the cases were insufficiently documented.
- Cases recorded in South Africa: a rash and a case of diarrhoea; Switzerland: a decrease in anti-vitamin K activity and a case of abdominal pain; and Malaysia: a *pityriasis rosea*. Causality was not established for these reports.

The searches conducted on ToxNet and the WHO site did not lead to the identification of any other reports of adverse effects associated with the consumption of spirulina.

3.2.5. Analysis of the literature data that might explain the adverse effects observed in nutrivigilance

3.2.5.1. Preclinical data

- **Spirulina**

Spirulina is neither mutagenic nor genotoxic (Gershwin and Belay 2008, Marles *et al.* 2011). It has been the subject of very numerous preclinical studies, mainly in rodents (mice, rats), with oral treatments at high doses (from 250 mg/kg/d to 10 g/kg/d, or even 30 g/kg/d of dried spirulina, or feed supplemented up to 30% *ad libitum*), for durations from 5 days to 6 months. Several studies have used pathological or toxicological models and compared two groups: one receiving spirulina and a control group. These studies did not show a deleterious effect of spirulina on food or drink intake, growth, the animals' state of health, reproductive functions (Pankaj 2015), or any teratogenic effect (Hutadilok-Towatana *et al.* (2008) and see for review: Gershwin and Belay (2008), Holman and Malau-Aduli (2013), Kay and Barton (1991) and Martinez-Galero *et al.* (2016)). A review devoted to the use of spirulina as a feed additive for livestock animals did not identify any problems of toxicity (Holman and Malau-Aduli 2013).

In addition, the "*United States Pharmacopoeia Safety Evaluation*" working group concluded as to a lack of toxicity, justifying a Generally Recognised As Safe (GRAS) status for spirulina at the usual consumption doses.

- **C-phycocyanin**

The no observed adverse effect level (NOAEL) of C-phycocyanin was assessed at 5 g/kg by the oral route and 70 mg/kg intraperitoneally.

In rats, C-phycocyanin administered *per os* up to the dose of 5 g/kg induced neither clinical signs nor mortality. Administration of 0.5 to 4 g/kg/d in food for 14 weeks did not alter food intake or body mass, or serum or haematological parameters, compared to untreated animals (Akhilender Naidu *et al.* 1999).

3.2.5.2. Clinical data

Forty-one clinical studies have been identified that assess the effects of spirulina, in particular as a food supplement, in diverse populations (sub-Saharan African, Asian, Caucasian; men and women) (see for review Gershwin and Belay (2008), Marles *et al.* (2011), Kay (1991)). When specified, the "spirulina" or "*S. platensis*" was consumed either directly, mixed with food, or in the form of food supplements. These clinical studies focused on between 15 and 500 patients. The target populations consisted of healthy or sick individuals, usually adults. They were in general placebo-controlled, and were not all randomly distributed. The studies did not give any particular specifications for these products. They were primarily interested in the effectiveness of spirulina,

and adverse effects were not specifically identified. The spirulina appeared to be well tolerated, and the few comparisons of adverse effects (mainly digestive disorders and headaches) with the placebo groups did not show any significant differences with regard to severity or incidence.

Spirulina (2 to 10 g/d and up to 19 g/d of dried spirulina) taken over long periods of time (up to 12 months), by healthy (children, adults, the elderly) and sick individuals (chronic hepatitis, HIV/AIDS, nephrotic syndrome), had no effects likely to be correlated with cases from nutrivigilance (Cingi *et al.* 2008, Johnson *et al.* 2016, Kalafati *et al.* 2010, Marcel *et al.* 2011, Mazokopakis *et al.* 2014, Miczke *et al.* 2016, Misbahuddin *et al.* 2006, Nakaya, Homma, and Goto 1988, Ngo-Matip *et al.* 2015, Park *et al.* 2008, Samuels *et al.* 2002, Selmi *et al.* 2011, Simpore *et al.* 2006, Torres-Duran, Ferreira-Hermosillo, and Juarez-Oropeza 2007, Torres-Durán *et al.* 2012, Winter *et al.* 2014, Yakoot and Salem 2012, Yamani *et al.* 2009, Marles *et al.* 2011). A study of the effects of a spirulina extract with a high phycocyanin content on coagulation parameters and platelet aggregation did not identify any anomaly (Jensen *et al.* 2016).

In studies conducted on cohorts of HIV-positive patients (17 to 170 subjects per study), there was no observed interaction between spirulina and antiretrovirals (Marcel *et al.* 2011, Ngo-Matip *et al.* 2015, Simpore *et al.* 2006, Winter *et al.* 2014, Yamani *et al.* 2009). A Cochrane meta-analysis relating to nutritional interventions in patients with HIV concluded that there was no significant difference in clinical, anthropometric and immunological outcomes between the groups receiving spirulina and control groups (placebo), in adults or children, as well as an absence of adverse effects (Grobler *et al.* 2013).

There are insufficient clinical data available from which to establish a spirulina dose not to be exceeded. Taking 10 g/d of dried spirulina does not seem to cause significant adverse effects in adults.

Like the reports analysed in nutrivigilance, the cases reported in the literature also showed primarily allergic, rheumatic or liver effects.

Clinical studies have reported a few adverse effects associated with the consumption of spirulina, such as digestive disorders and headaches. However, many studies have shown no such effects. Specific individual susceptibility/hypersensitivity (probably of immunological origin) to this cyanobacterium is *a priori* rare, which would probably explain why it was not detected during clinical studies involving too few subjects for this.

Contamination during production and/or packaging cannot be ruled out as an aetiology for the cases of adverse effects reported after consumption of spirulina. This underlines the importance of observing the good hygiene practices mentioned in Section 3.1.2.

3.3. Vulnerable populations and at-risk situations

Two studies conducted in undernourished/malnourished infants and children (one including 23 children from 2 to 13 years, and the other 550 children under 5 years of age) at doses of 5 g/d showed no adverse effect (Samuels *et al.* 2002, Simpore *et al.* 2006). The cases of anaphylaxis reported in Section 3.2 were observed in two teenagers with an allergic predisposition.

Consumption of spirulina containing amino acids, in particular phenylalanine, is not recommended in individuals suffering from phenylketonuria.

Because of the cases of allergies reported following consumption of spirulina, such consumption is not recommended in individuals with an allergic predisposition.

3.4. Conclusion and recommendations of the CES and the WG

Spirulina is an ancient traditional food in several countries. In France, it is found on the market in the form of a conventional food (alone or as an ingredient) or as a food supplement.

Several cases of adverse effects occurring following the intake of food supplements containing spirulina have been brought to the attention of the nutrivigilance and toxicovigilance schemes, and the vigilance systems of several Member States of the European Union, and Canada. The doses consumed in these cases are not precisely known. Some of the cases have also been the subject of publications in scientific journals. The effects vary greatly, with digestive disorders being the most frequently reported.

Spirulina has undergone preclinical and clinical studies. The preclinical data have not shown spirulina to be toxic at high doses (for administered doses up to 30 g/kg/d, or even *ad libitum* in mice). Among the many clinical studies conducted with doses of up to 19 g/d of dried spirulina, only a few adverse effects related to its consumption have been reported, such as digestive disorders and headaches. Nevertheless, too few subjects are included in these studies to be able to demonstrate rare effects such as individual susceptibility/hypersensitivity.

Products containing spirulina can be contaminated by cyanotoxins, bacteria or trace metal elements.

Most products containing exclusively spirulina (*Arthrospira* spp.) that have undergone contamination studies do not have a worrying level of cyanotoxins, unlike other cyanobacteria (*Aphanizomenon flos-aquae*). Nevertheless:

- there are insufficient data available in the literature to be able to rule out the potential production of certain cyanotoxins (hepatotoxic microcystins) by *Arthrospira* spp.;
- contamination by other cyanotoxin-producing cyanobacteria is possible in traditional or industrial settings.

Bacterial contamination at concentrations exceeding the usual food standards seems to be rare for finished products. Nevertheless, contamination can occur during handling (in particular during harvesting, washing, drying, storage or packaging).

The presence of trace metal elements (lead, mercury, arsenic) at concentrations exceeding the limits laid down by the European Union or the specifications of the *USP Dietary Supplements Compendium* has been reported in commercial samples of spirulina.

In conclusion, the CES stresses that:

- Apart from the risk of contamination, spirulina does not seem to present a health risk at low doses (up to several grams per day).
- The clinical data have not shown any vulnerable populations or at-risk situations, with the exception of individuals suffering from phenylketonuria or with an allergic predisposition. The CES emphasises the existence of reported cases of muscle or liver damage following consumption of spirulina in the form of a food supplement, without it being possible to characterise the relationship with the dose and the duration of consumption.
- Consumption of 5 g/d of spirulina (maximum quantity recommended by certain food supplements) provides from 7 to 8.5 mg of beta-carotene, whereas the maximum recommended daily intake of beta-carotene from food supplements has been estimated at 7 mg/d.

In light of the risk of contamination, in particular by TMEs and cyanobacteria, the CES insists on the importance of the quality of the water used for production, the selection of the inoculum and the control of the different stages of production.

With regard to microcystins, the WHO has established a TDI of 0.04 µg/kg/d. The CES therefore considers it necessary to establish a threshold for microcystins in food supplements containing spirulina that takes into account other dietary intakes of microcystins.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety adopts the recommendations of the Working Group on "Nutrivigilance" and the Expert Committee on “Human Nutrition”.

The Agency emphasises that products containing spirulina can be contaminated by cyanotoxins, bacteria or trace metal elements. In this context, the Agency recommends that consumers should favour supply channels with the best oversight by the public authorities (compliance with French regulations, traceability and identification of the manufacturer).

In addition, ANSES considers it necessary to conduct an expert appraisal to establish a threshold for microcystins in food supplements containing spirulina that takes into account other dietary intakes of microcystins, and to reassess the limit established for food supplements containing Klamath alga.

Apart from the risk of contamination, spirulina does not seem to present a health risk at low doses (up to several grams per day in adults). Nevertheless, ANSES considers that clinical studies should be undertaken to establish a maximum daily dose, with sufficient numbers of subjects to detect rare effects such as a specific individual susceptibility/hypersensitivity to this cyanobacterium.

In light of the characteristics of spirulina and the adverse effects reported, ANSES advises against the consumption of these food supplements by individuals suffering from phenylketonuria or with an allergic predisposition, or a muscle or liver vulnerability.

In addition, the Agency emphasises that spirulina is not a reliable source of vitamin B12 for populations avoiding consumption of products of animal origin, as it is mostly in the form of an inactive analogue.

ANSES reminds healthcare professionals of the need to report to its nutrivigilance scheme any adverse effects likely to be associated with the consumption of food supplements about which they become aware.

Lastly, ANSES emphasises the value of setting up a joint international project on the monitoring of adverse effects associated with the consumption of food supplements.

KEYWORDS

Nutrivigilance, effets indésirables, compléments alimentaires, spiruline, *Arthrospira* spp., cyanobactéries, microcystines

Nutrivigilance, adverse effects, food supplements, spirulina, *Arthrospira* spp., cyanobacteria, microcystins

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ANNEX 1

Presentation of participants

PREAMBLE: The expert members of the Expert Committees and Working Groups or designated rapporteurs are all appointed in a personal capacity, *intuitu personae*, and do not represent their parent organisation.

NUTRIVIGILANCE WORKING GROUP

Chair

Mr Alexandre MACIUK – University Lecturer (Paris-Sud University) – Speciality: pharmacognosy

Members

Ms Catherine ATLAN – University Lecturer – Hospital Practitioner (Luxembourg Hospital Centre) – Specialities: metabolic diseases, nutrition and endocrinology

Mr Alain BOISSONNAS – Retired, University Professor – Hospital Practitioner (University Hospital Paris-Sud) – Speciality: general medicine

Ms Sabrina BOUTEFNOUCHET – University Lecturer (Paris-Descartes University) – Speciality: pharmacognosy

Mr Pierre CHAMPY – University Professor (Paris-Sud University) – Speciality: pharmacognosy

Mr Pascal CRENN – University Professor – Hospital Practitioner (Raymond Poincaré Hospital) – Speciality: hepato-gastroenterology

Mr Thierry HENNEBELLE – University Professor (Lille II University) – Speciality: pharmacognosy

Ms Raphaële LE GARREC – University Lecturer (University of Western Brittany) – Speciality: toxicology

Mr Jean-Marie RENAUDIN – Hospital Practitioner (Emilie Durkheim Hospital Centre) – Speciality: allergology

Ms Dominique Angèle VUITTON – Retired, University Professor – Hospital Practitioner (University of Franche Comté) – Specialities: allergology, hepato-gastroenterology

Mr Bernard WENIGER – Retired, University Lecturer (Strasbourg University) – Speciality: pharmacognosy

Mr Jean-Fabien ZAZZO – Hospital Practitioner (Antoine Béclère Hospital) – Specialities: general medicine, nutrition

RAPPORTEURS

Mr Xavier BIGARD – Honorary Professor – (Military Health Service) – Specialities: physiology of exercise, muscular and nutrition biology

Ms Sabrina BOUTEFNOUCHET – University Lecturer (Paris-Descartes University) – Speciality: pharmacognosy

Mr Pierre CHAMPY – University Professor (Paris-Sud University) – Speciality: pharmacognosy

EXPERT COMMITTEE

The work that is the subject of this opinion was monitored and adopted by the following CES:

CES on "Human Nutrition" – 2015-2018

Chair

Mr François MARIOTTI – Professor (AgroParisTech) – Specialities: metabolism of proteins, amino acids, nutritional requirements and recommendations, postprandial metabolism, cardiometabolic risk

Members

Ms Catherine ATLAN – Doctor (Luxembourg Hospital Centre) – Specialities: endocrinology, metabolic diseases

Ms Catherine BENNETAU-PELISSERO – Professor (Bordeaux Sciences Agro) – Specialities: phyto-oestrogens, isoflavones, endocrine disruptors, bone health

Ms Marie-Christine BOUTRON-RUAULT – Research Director (CESP Inserm) – Specialities: nutritional epidemiology and cancer, digestive system

Mr Jean-Louis BRESSON – University Professor – Hospital Practitioner (AP-HP Necker Hospital – Sick Children, Centre for Clinical Investigation 0901) – Specialities: epidemiology, immunology, infant nutrition, pregnant women and proteins

Mr Olivier BRUYERE – University Professor (University of Liège) – Specialities: epidemiology, public health, osteoporosis

Ms Blandine DE LAUZON-GUILLAIN – Research Manager (Inserm, CRESS, Villejuif) – Specialities: epidemiology, infant nutrition, nutrition of pregnant and breastfeeding women, public health

Ms Anne GALINIER – University Lecturer – Hospital Practitioner (Paul Sabatier University – Toulouse University Hospital) – Specialities: metabolism of adipose tissue/obesity, pathophysiology

Mr Jean-François HUNEAU – Professor (AgroParisTech) – Speciality: human nutrition

Ms Emmanuelle KESSE-GUYOT – Research Director (INRA, UMR Inserm U1153/INRA U1125/CNAM/University of Paris 13) – Specialities: epidemiology, nutrition and pathologies, nutrition and public health

Ms Corinne MALPUECH-BRUGERE – University Lecturer (University of Auvergne) – Speciality: nutrition of pathologies, metabolism of macro- and micronutrients

Ms Catherine MICHEL – Research Manager (INRA, UMR INRA/University, Nantes) – Specialities: infant nutrition, intestinal microbiota, colic fermentation, prebiotics

Ms Beatrice MORIO-LIONDORE – Research Director (INRA Lyon) – Specialities: human nutrition, energy metabolism

Ms Jara PEREZ-JIMENEZ – Contract Researcher (ICTAN – CSIC, Madrid) – Specialities: micro-constituents, nutrition and pathologies, bioavailability

Mr Sergio POLAKOFF – Research Manager (INRA Clermont-Ferrand/Theix) – Specialities: nutrition and pathologies, nutrition and public health, energy metabolism

Mr Jean-Marie RENAUDIN – Hospital Practitioner (Emilie Durkheim Hospital Centre) – Specialities: allergology

Ms Anne-Sophie ROUSSEAU – University Lecturer (University of Nice Sophia Antipolis) – Specialities: nutrition and physical activity, bioavailability, oxidative stress

Mr Luc TAPPY – University Professor – Hospital Practitioner (University of Lausanne) – Specialities: endocrinology, metabolism of carbohydrates

Mr Stéphane WALRAND – Research Director (INRA Clermont-Ferrand/Theix) – Specialities: pathophysiology, protein metabolism and amino acids

ANSES PARTICIPATION

Scientific coordination

Ms Charlotte LEGER – Scientific and Technical Project Leader – Risk Assessment Department

Scientific contribution

Ms Gwenn VO VAN-REGNAULT – Nutrivigilance Project Officer – Risk Assessment Department

Ms Irène MARGARITIS – Head of the Nutritional Risk Assessment Unit – Seconded University Professor (University of Nice Sophia Antipolis) – Risk Assessment Department

Ms Nathalie ARNICH – Deputy Head of the Foodborne Risk Assessment Unit – Risk Assessment Department

Administrative and secretarial assistance

Ms Virginie SADE – Risk Assessment Department

HEARINGS WITH EXTERNAL EXPERTS

Federation of French Spirulina Producers (FSF)

Ms Nathalie DE POIX – President of the FSF

Mr Emmanuel GORODETZKY – Administrator and founding member of the FSF

Mr Vincent RIOUX – Administrator and founding member of the FSF

ANNEX 2

Report of the hearing with the Federation of French Spirulina Producers (FSF)

ANSES would like to thank the participants for making themselves available, and reiterates the context of this hearing. The Agency issued an internal request following the receipt of reports of different types of adverse effects suspected of being associated with the consumption of food supplements containing spirulina. The expert appraisal under way involves an analysis of the reports received by the Agency and of the existing literature. In order to collect information on the methods of production in France, ANSES wished to meet with the Federation of French Spirulina Producers (referred to below as "the Federation").

With a view to preparing for the discussions, the Federation was sent several questions, and was invited to add any other information it felt should be brought to the attention of the Agency.

ANSES wished to know how many members were in the Federation and how many non-member producers there were, if applicable, as well as the annual tonnage of spirulina produced in France.

The Federation has 160 members, all based in metropolitan France; no applications for membership have been made in the overseas territories. It estimates that there are around 50 non-member producers. The producers that are members of the Federation produce a total of 30 to 35 tonnes of spirulina per year. This supply is not enough to meet demand.

ANSES asked about the production conditions and whether there were any specifications or a charter.

The Federation has a charter that makes reference to a guide to good hygiene practices (GGHP). This guide states that production should take place in ponds inside a greenhouse. There are currently several types of installation. The spirulina harvesting, pressing, shaping and drying operations take place either in the production greenhouse or in a separate room. The Federation members do not use reactors for production.

The Federation is awaiting validation of the GGHP. It offers training in good hygiene practices for new producers and a continuous training programme made up of modules on technical aspects and traceability. Thirty producers have already followed training of this type.

ANSES wished to know whether the ponds were fully renewed or whether cultivation took place continuously.

The Federation stipulates full or partial renewals, in winter, as well as measures to verify the quality of the media. When the growing media is renewed, the basins are cleaned and disinfected.

ANSES asked about the origin of the strains and the frequency of subculturing.

Several strains of the species *Arthrospira platensis* are grown; their name depends on the geographical region where the spirulina was collected. In France, the cultivated strains come from Lake Paracas (Peru), Lake Lonar (India) or from Ethiopia, Chad or Madagascar.

The Laboratoire Limnologie (Rennes) isolates strains at the request of the producer. Biology training is available and there are plans with the Centre for the Study and Promotion of Algae

(CEVA) to establish a training programme to teach producers how to isolate and maintain their strains.

ANSES asked whether quality controls and audits were carried out, how often, and at what stage of production.

Quality controls (microbiological analyses, tests for heavy metal and iodine content in the finished product, screening for microcystins, pH controls, etc.) are performed by the producers at several stages of production. All of the analyses are performed in the Federation's partner laboratories (AQMC, Limnologie). The frequency of analysis is determined by the producer depending on the volume of production, batches identified and results obtained. Every year, the Federation meets to share the results of these controls with the partner laboratories.

The Federation added that the Laboratoire Limnologie sets a level of spirulina biomass at 99.6% and that it is possible to find other cyanobacteria during controls. The Laboratoire Limnologie has a photo library of the other cyanobacteria in order to identify them. If any potentially toxic cyanobacteria are identified, the producer tests for microcystins. The results from contaminant screening are good and in a range of +/- 10% (for microcystins in the finished products: out of 39 ELISA analyses in 2016, there was a 38% detection rate (< 0.5 µg/g)). In the absence of regulation, the GGHP proposes the limit of 1 µg/g. This limit remains to be validated by the authorities when reviewing the GGHP. According to the recommendations of the GGHP, batches above 1 µg/g should not be placed on the market.

In addition, five voluntary audits have already been performed, to monitor implementation of the GGHP.

ANSES wished to know what water was used, what treatments are applied to the water used, and the origin of the inputs.

The farms are supplied with clean or drinking water. The GGHP specifies the analyses to be performed. Drinking water is mandatory from the pressing stage. Water from outside the distribution system is treated by different means (e.g. active carbon filter, UV filters, etc.). The GGHP refers to Council Directive 98/83/EC of 3 November 1998 on drinking water and Regulation (EC) 852/2004, which defines clean water.

The inputs currently used by the Federation are inputs used in agriculture. The Federation is currently testing new inputs of organic origin.

ANSES questioned the Federation about the means to combat potential sources of contamination.

The GGHP identifies the risks: chemical, microbiological, physical and allergens. The guide identifies several operational prerequisites to be monitored during production (e.g. compliance with the cold chain, presence of debris from glass, etc.). Bacterial contamination (*Listeria*, *Salmonella*, *Staphylococcus*, thermotolerant coliforms, total flora) is screened for, with application of the thresholds proposed in the GGHP (CSHPF Opinion, Regulation (EC) No 2073/2005 as amended, and criteria proposed by the FSF). Several control measures are proposed by the Federation. Production does not require any pesticides or medicinal products, or any other products, treatments or preservatives.

ANSES asked about the status of products from the Federation's producers, whether the spirulina produced was exported and what the distribution channels were.

The Federation's members produce strands, flakes and fresh biomass, which fall within the scope of food. However, the Federation is not involved in the marketing of spirulina in the form of tablets, capsules or powders intended for the manufacture of food supplements. Some members do however get accredited laboratories to make customised tablets. The MAs and the manufacture of tablets, capsules or powder are not supervised by the Federation.

In addition, spirulina produced by the Federation's members is not exported but can be sold by cross-border producers. Short distribution channels (direct sales) are preferred. Clients are primarily individuals.

ANSES wished to know whether there was production of extracts and whether, if applicable, this was carried out by the spirulina producers.

The Federation stated that there was no production of extracts among its members.

ANSES asked the Federation whether it was aware of consumer expectations.

The Federation does not resort to advertising, since health claims relating to spirulina are not authorised. It only provides information on the composition of the spirulina produced and explains to consumers that it is a food and not a drug, in order to provide some perspective for the health benefits promoted in the press.

ANSES wished to know whether the Federation had links with other professional organisations.

The Federation's main point of contact is the Directorate for Maritime Fisheries and Aquaculture. The Federation also liaises with the Directorate General for Education and Research, and with the Directorate General for Food, in particular regarding the GGHP. There is a standardisation project at European level on algae in general; the Federation may therefore be required to liaise with AFNOR. The Federation is working with the FNAB, in the framework of the organic agriculture specifications, with the CEVA in the framework of the Special Funds for Agricultural and Rural Development (CASDAR) on the topic of strains, and also with universities and research centres.