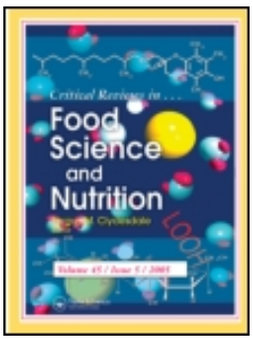

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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To cite this article: Robin J. Marles , Marilyn L. Barrett , Joanne Barnes , Mary L. Chavez , Paula Gardiner , Richard Ko , Gail B. Mahady , Tieraona Low Dog , Nandakumara D. Sarma , Gabriel I. Giancaspro , Maged Sharaf & James Griffiths (2011) United States Pharmacopeia Safety Evaluation of Spirulina, Critical Reviews in Food Science and Nutrition, 51:7, 593-604, DOI: 10.1080/10408391003721719

To link to this article: https://doi.org/10.1080/10408391003721719

Published online: 27 Jul 2011.

Critical Reviews in Food Science and Nutrition, 51:593–604 (2011)

ISSN: 1040-8398 print / 1549-7852 online
DOI: 10.1080/10408391003721719

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United States Pharmacopeia Safety Evaluation of Spirulina

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The Dietary Supplements Information Expert Committee (DSI-EC) of the United States Pharmacopeial Convention (USP) reviews the safety of dietary supplements and dietary supplement ingredients for the purpose of determining whether they should be admitted as quality monographs into the United States Pharmacopeia and National Formulary (USP–NF). *The United States Food and Drug Administration (FDA) has enforcement authority to pursue a misbranding action in those instances where a dietary supplement product indicates that it conforms to USP standards but fails to so conform. Recently DSI-EC undertook a safety evaluation of spirulina, a widely used dietary ingredient. DSI-EC reviewed information from human clinical trials, animal studies, and regulatory and pharmacopeial sources and analyzed 31 adverse event reports regarding spirulina to assess potential health concerns. At the conclusion of this review, DSI-EC assigned a Class A safety rating for* Spirulina maxima *and* S. platensis*, thereby permitting the admission of quality monographs for these dietary supplement ingredients in* USP–NF. *DSI-EC continually monitors reports concerning the safety of dietary supplements and dietary supplement ingredients for which USP dietary supplement monographs are developed. The DSI-EC may revisit the safety classification of spirulina as new information on this dietary ingredient becomes available.*

Keywords Spirulina, *Arthrospira,* blue-green algae, cyanobacteria, USP, dietary supplements

INTRODUCTION

USP was founded by physicians in 1820 as an independent, science-based, not-for-profit, standards-setting organization for drugs. It has evolved as an organization setting standards for modern prescription drugs, biologics, excipients, dietary supplements, and food ingredients. Two of its principal publications, the *United States Pharmacopeia* and the *National Formulary* (collectively, *USP–NF)*, are recognized in the federal Food, Drug, and Cosmetic Act (FDCA) as official compendia in the United States (US) (Bhattacharya et al., 2004; Schiff et al., 2006). USP's documentary standards (monographs and allied General Chapters) and reference materials, also called official USP Reference Standards (RS), are used not only in the US but also in approximately 130 other nations worldwide. USP's standards-setting body is the Council of Experts, which currently has five Expert Committees that create quality standards for dietary supplements (DS) and dietary ingredients (DI), as defined in FDCA. These are: the DSI-EC; DS Performance Standards; DS Botanicals; DS General Chapters; and DS Nonbotanicals Expert Committees. Beyond USP's documentary standards and RS, USP has established DS and DI Verification Programs. These programs involve a current Good Manufacturing Practices (cGMPs) audit, quality system review, and product testing performed by USP staff scientists to ascertain whether DS and DI manufacturers participating in the voluntary verification program conform to *USP–NF* quality standards and can thereby be permitted to use the "USP Verified" mark on the product label (Atwater, 2003; Atwater et al., 2005).

Unless a product is a new dietary ingredient, the Dietary Supplements Health and Education Act (DSHEA, 1994) amendment to FDCA does not require FDA approval for dietary supplements before marketing. For new dietary ingredients that were

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not legally marketed in the US before 15 October 1994, DSHEA requires the manufacturer or distributor to submit a notification to FDA 75 days before "introducing or delivering for introduction into interstate commerce a dietary supplement that contains a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered . . . [and] information including any citation to published articles that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such dietary ingredient will reasonably be expected to be safe."[1] DSHEA also stipulates that if a DS is 1) covered by the specifications (tests, procedures, and acceptance criteria of a monograph) of an official compendium, 2) is represented as conforming to the specifications of an official compendium, but 3) fails to so conform, then the supplement is considered to be misbranded under FDCA [§403(s)(2)(D)] (DSHEA, 1994). However, §403(s)(2)(D) of FDCA makes compliance with *USP–NF* specifications strictly voluntary, and DS manufacturers can decide whether or not to place USP–NF on the product label. This is in contrast to prescription drugs, for which conformance with USP–NF standards is mandatory, whether products are labeled USP–NF or not. Nonetheless, §403(s)(2)(D) of FDCA provides legal recognition for USP–NF standards for dietary supplements, creating the possibility of FDA enforcement for misbranding against those manufacturers who claim compliance with these standards but fail to so conform.

To select and prioritize candidate dietary ingredients for monograph development, USP has developed an *Admission Criteria and Safety Classification for Dietary Supplements Guideline (Guideline)* (USP, 2009; Schiff et al., 2006) that evaluates parameters for admission: 1) apparent efficacy or a presumptive belief in some beneficial activity as evidenced by a long history of use; 2) demand, or the extent of use by the public sector; 3) public protection, indicating interest by a regulatory agency; 4) feasibility, suggesting the likelihood that the ingredient could meet compendial criteria; 5) compendial presence, demonstrated by the existence of monographs in other official compendia; and 6) safety, as indicated by a long history of use (or recent data indicating no safety concerns).

As an element of the *Guideline*, DSI-EC evaluates the DS safety profile of a dietary supplement ingredient to determine its admission in *USP–NF* as a quality monograph. DSI-EC conducts extensive safety reviews of the selected dietary ingredient, analyzing information from human clinical case reports, adverse event reports (AERs), animal pharmacological and toxicological data, historical use, regulatory status, and global contemporaneous extent of use (detailed in USP, 2009; Sarma et al., 2008; Mahady et al., 2008). Following a thorough review of this information, DSI-EC assigns one of the following safety and admission classifications to the DS articles for monograph development: 1) Class A: Admitted into *USP–NF* or 2) Class B: Not admitted into *USP–NF*. Under Class A, articles for which the available evidence does not indicate a serious risk to health (defined in USP, 2009; EMEA, 1995) or other public health concern are approved for the development of quality monographs and are admitted in *USP–NF*. Following this decision, other USP DS Expert Committees may consider setting quality standards, and the DS or DI may be eligible to participate in USP's Verification Programs. DSI-EC's safety review of spirulina was conducted in accordance with USP's *Guideline*.

Several authors have suggested the need for quality standards for spirulina (Rellan et al., 2009; Eisenbrand et al., 2008). Following a request from a monograph sponsor (i.e., a company that has volunteered to help develop a monograph by providing validated data for quality specifications), DSI-EC, working with USP staff, reviewed the safety of spirulina to determine whether a spirulina quality monograph could be admitted into *USP–NF*. This article summarizes DSI-EC's safety review of spirulina.

PRODUCT DESCRIPTION

The name *Spirulina* refers to a large number of eubacterial species that belong to the phylum *Cyanobacteria* (Muhling et al., 2006), family *Spirulinaceae* or *Pseudanabaenaceae* (NCBI, 2009). Spirulina also are known as blue-green algae, but they are in reality prokaryocytes (they lack the nuclei and other cellular organization of eukaryotes such as algae). Thirty-five species of spirulina have been identified (Dillon et al., 1995). The most commonly used spirulina (also called *Arthrospira*) species in dietary supplements are *S. maxima* (Setchell and Gardner) Geitler, *S. platensis* (Nordstedt) Geitler, and *S. fusiformis* Voronikhin (McGuffin et al., 2000).

Spirulina use has been widely documented since the 16th Century (Ciferri, 1983). Spirulina contains several nutrients, including about 65% protein, B-complex vitamins, phycocyanin, chlorophyll, β-carotene, vitamin E, superoxide dismutase, and numerous minerals (Wang et al., 2008; Dillon et al., 1995; Lumsden and Hall, 1974). High concentrations of polyunsaturated fatty acids and γ-linolenic acid also are present (Otles and Pire, 2001). Spirulina is the first prokaryote found to contain stable, easily extractable ferredoxin (Tanaka et al., 1975). Phycocyanin is the most abundant protein-bound pigment in Cyanobacteria and accounts for more than 20% of its dry weight (Romay et al., 1998). In view of its nutrient content, it is considered suitable as a functional food (Park et al., 2008).

Several spirulina-based products are available in the US as food or dietary supplements and in different forms, including powder or capsules (NBJ, 2009; DSLD, 2009). Although historical accounts (Ciferri, 1983) indicate consumption of spirulina (as Dihé in Africa) at 10 to 40 g, the contemporary typical intake of spirulina is 1–5 g daily before meals (Fetrow and Avila, 1999; NLM, 2009; Gilroy et al., 2000; FDA, 2003). Recent clinical studies (Yamani et al., 2009) indicate that an intake of about 10 g of spirulina per day for 6 months does not induce adverse effects.

Other species of Cyanobacteria that are sold for consumption as dietary supplements or foods include *Aphanizomenon*

[1]See *Title 21 Code of Federal Regulations* §190.6(a) (2009).

flos-aquae (L.) Ralfs ex Barnet et Flahault and *Nostoc ellipsosporum* Rabenhorst ex Bornet et Flahault, both in the family Nostocaceae (NCBI, 2009). Correct identification of the cyanobacterial material is an important distinction because of the well-established presence of toxins in certain genera, including Aphanizomenon and Microcystis, (e.g., Schaffer et al., 1999) and the apparent absence of toxins in other genera, such as Spirulina. Cyanobacteria either are harvested from natural, warm, alkaline waters or are grown under controlled conditions. Richmond (1999) detailed the systems for mass production of microalgae outdoors. Toxin concentrations in genera other than Spirulina are affected by environmental factors such as exposure to sun, depth of the water in which the organisms live, and the types of minerals in the water. Toxin concentrations fluctuate with environmental changes and are not predictable. Without scientific testing, users have no reliable way to detect the presence or concentration of toxins. Several useful reviews of cyanobacterial toxins are available (Health Canada, 2008a; Purdue, 2007; Dietrich and Hoeger, 2005; Schaffer et al., 1999). The proposed standards for the USP quality monograph for spirulina and detailed notes about microcystins are provided in the section titled "Contaminants" below.

METHODS

DSI-EC reviewed the following resources to conduct its safety review: spontaneous AERs from FDA's MedWatch program, the Canada Vigilance Adverse Reaction Online Database from the Canadian Department of Health, and the Australian Adverse Drug Reaction Reporting System from Australia's Therapeutic Goods Administration (search duration mentioned below). In addition, USP staff searched PubMed and TOXNET (1966 to October 2009) to retrieve clinical case reports and animal pharmacological or toxicological information. DSI-EC also collected information concerning the regulatory status and pharmacopeial standards of spirulina in other countries. The principles of data collection, evaluation, and integration are detailed in the *Guideline* (USP, 2009) and in the Institute of Medicine *Framework for Evaluating Safety of Dietary Supplements* (IOM, 2005). Limitations of the dietary supplement AERs (detailed in Gardiner et al., 2008) are considered in the analysis of the reports. The safety classification for spirulina is based on the expert opinion of DSI-EC to determine admission to *USP–NF* as a quality monograph. The proposed USP standards for spirulina are developed in accordance with principles detailed in Schiff et al. (2006).

SAFETY REVIEW

DSI-EC found numerous experimental and clinical studies that investigated the efficacy of spirulina, but the current review focuses only on reports that concern the safety of spirulina. This report does not include a review of the efficacy studies from clinical reports or animal models. The primary objective of this review is to identify signals of safety concern associated with the use of spirulina. This section includes analyses of reports regarding the following aspects of safety:

- Human data—AERs
  - Clinical case reports
  - FDA MedWatch reports
  - Canada Vigilance Program reports
  - Australian Therapeutic Goods Administration (TGA) reports
  - UK Medicines and Healthcare products Regulatory Agency reports
  - Uppsala Monitoring Centre
- Animal pharmacological data
- Supplement–drug interactions
- Contaminants such as microcystin, heavy metals, and microbial organisms
- Regulatory status.

Safety information for spirulina-based products follows.

Clinical Case Reports

Although spirulina has a long history of recorded use (Ciferri, 1983), a review of the literature up to and including October, 2009, revealed no systematic clinical safety evaluation in the public record. This is surprising because historically spirulina was cultivated from wild open lakes, and contamination with other blue–green algae that produce microcystins was a matter of serious concern. In the present review, clinical case reports of adverse effects of spirulina published in peer-reviewed scientific journals were identified using the PubMed database (available at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed). The search covered the period from 1966 to October, 2009. The following search terms (number of references in parenthesis) were used: spirulina (777); arthrospira (78); spirulina AND adverse effects (72); spirulina AND toxicity (62); spirulina AND clinical trial (28); spirulina AND toxic effects (17); and spirulina AND safety (9). Nonduplicate reports were collected from TOXNET (http://toxnet.nlm.nih.gov/index.html). Most references corresponding to terms "adverse effects," "toxicity," "toxic effects," and "safety" did not directly correspond to the main topic of the search. Clinical studies of spirulina were conducted primarily to evaluate its nutritional or biological activities, and, accordingly, little safety information was retrievable from these studies. A review of the reports that directly related to safety concerns in clinical case reports indicated 3 clinical observations recording adverse events associated with use of spirulina products.

Mazokopakis et al. (2008) reported a case of acute rhabdomyolysis in a 28-year-old man who was taking spirulina tablets as a dietary supplement (at the manufacturer's recommended intake of 3 g/day) during the previous 1-month period. The patient

was reportedly not taking other medications and did not use alcohol or illicit drugs. Hematological tests were indicative of rhabdomyolysis, showing elevated creatinine kinase concentrations and elevated concentrations of other muscle enzymes such as myoglobin, ALT, AST, LDH, and aldolase. The patient was hydrated for 4 days and then discharged. One week later, he remained free of symptoms, and laboratory findings were normal. The authors hypothesized that under certain conditions blue-green algae could produce a neurotoxin called BMAA (β-*N*-methylamino-*L*-alanine), which can cause a neurodegenerative disease (amyotrophic lateral sclerosis–parkinsonism–dementia complex) but did not provide any evidence for the presence of BMAA in the product used. Although BMAA is known to be produced by other cyanobacterial species such as *Nostoc* (Johnson et al., 2008), its production in spirulina has not been reported before. Further, a recent study has shown preventive effects of spirulina on skeletal muscle damage under exercise-induced oxidative stress (Lu et al., 2006).

A. flos-aquae and *S. platensis* were implicated in a patient's diagnosis of dermatomyositis (Lee and Werth, 2004). The patient was a 45-year-old woman with a history of hypertension, chronic migraines, and fibromyalgia. The patient reportedly developed redness on her face and over the knuckles of her hands within 1 to 2 days following use of a supplement composed of organic cayenne pepper, methylsulfonylmethane (also known as MSM), *A. flos-aquae,* and *S. platensis*. She discontinued the supplement but a month later resumed it, along with another multi-ingredient product containing digestive enzymes. Four days after rechallenge with the supplement, she reported worsening of the rash, including extensive swelling of her face, eyes, and ears. Subsequent investigations revealed that her antinuclear antibody titer was 1:160, and a biopsy supported diagnosis of dermatomyositis. Later studies showed that the patient was heterozygous for the –308A tumor necrosis factor-α (TNF-α) promoter polymorphism, the phenotype of which is increased TNF-α production, which in turn may genetically predispose an individual to autoimmunity (Lee and Werth, 2004). DSI-EC views this case report as a classical example of idiosyncratic reaction. In individuals with a genetic predisposition, a supplement such as spirulina that is claimed to be an immunostimulant can help precipitate an autoimmune disorder such as dermatomyositis. Further, the use of more than one product with multiple ingredients complicates causality assignment.

Iwasa et al. (2002) reported the observation of elevated liver enzyme concentrations in a 52-year-old Japanese man who used a spirulina product for 5 weeks. The patient had a history of hypertension, hyperlipidemia, and type 2 diabetes mellitus, and had taken simvastatin and amlodipine for 7 months. Unfortunately the paper did not discuss the well-documented possibility that a statin such as simvastatin could cause liver damage. The report concluded that the adverse reaction possibly was related to spirulina because the patient's liver enzyme concentrations improved after withdrawal of spirulina (positive dechallenge). However, all the patient's medications were withdrawn concurrently, so alternative explanations for the decline in liver enzyme concentrations cannot be excluded.

Table 1 Clinical trials of spirulina

Study Title	Study Details
Vitamin A value of spirulina carotenoids in humans	NCT # 00680277 Study design: Phase I and Phase II; Nonrandomized, Open-label, Uncontrolled, Single Group Assignment, Bioequivalence Study 5 g/day of spirulina for 8 weeks (subjects: N = 20) Study location / status: Tufts University/ Recruitment closed
Impact of *Spirulina platensis* supplementation on general health status of HIV-infected patients in Burkina Faso	ISRCTN# 83770226 Study design: Double-blind randomized controlled trial 5 g/day of spirulina for 12 months (subjects: N = 60) Study location / status: Burkina Faso/Recruitment closed (Simpore et al., 2005)

Clinical trials registries (www.clinicaltrials.gov/; http://www.controlled-trials.com/mrct/search.html; http://hnrim.nih.gov/; http://crisp.cit.nih.gov/; and www.who.int/ictrp/en) recorded 2 studies whose primary objective was evaluating the efficacy of spirulina. Safety and tolerability of spirulina are not reported in these studies. The Cochrane Library indexed 18 clinical trials (English reports) but no Cochrane Review for the term *spirulina*. The trials focused on efficacy investigations and reported few data relating to safety aspects. Study details for some clinical trials (Table 1) show that few registered clinical investigations are documented for spirulina and that no safety concerns were described in clinical studies.

FDA MedWatch AERs

The authors reviewed FDA MedWatch reports involving spirulina during the period from January, 2001, to July, 2009, and identified 79 nonduplicate reports. Of these, 38 involved concurrent treatment with ephedra. Because of earlier AERs associated with ephedra (Woolf et al., 2005), these 38 reports were excluded from the present analysis. In addition, because of the well-established presence of toxins in certain genera of cyanobacteria, including Aphanizomenon and Microcystis, AERs associated with products that contained these ingredients were excluded from analysis.

In total, 5 reports of liver damage and 8 reports of other adverse outcomes associated with use of spirulina were identified (Tables 2 and 3). The most common non-serious adverse events were nausea, diarrhea, vomiting, fatigue, headache, dizziness, itching, rash, and abdominal cramps.

As with many spontaneous AERs, several MedWatch reports lacked information about the quantity of spirulina used, duration of usage, patient history, and product quality. Further, these reports do not present a pattern of pathology. These reports were associated with different dietary supplements containing spirulina, and product information was not available for any of them so their spirulina content is unknown. If additional information becomes available, the causality relationship for spirulina in these reports may change. For example, MedWatch case report #16471 identifies the product simply as "blue-green algae" that

Table 2 Details of MedWatch cases reporting liver damage as the outcome

Case Number	Received Date	Pathology	Comments
# 14853	23 April 2001	Elevated liver function test values	No other details were recorded
# 15515	28 February 2002	Elevated liver enzymes, resulting in death	Significant medical history: Hepatitis A, B, C, alcohol usage, jaundice, and diabetes
# 67512	16 January 2004	Toxic hepatitis	The product contains more than 50 ingredients
# 98498	30 November 2007	Elevated liver enzymes	Patient was using Depo-Provera and several polyherbal and multivitamin combinations containing spirulina
# 110611	13 February 2009	Elevated liver enzymes, drug-induced lupus, hair loss, blot clot	This spontaneous report by a female patient indicated reactions upon use of a multi-ingredient product containing spirulina

was not analyzed. The neurological manifestations in this report are consistent with the ones produced by the neurotoxins anatoxin or saxitoxin, which indicates the possibility of contamination with other cyanobacteria such as Anabaena, Oscillatoria, or Aphanizomenon.

Summary Analysis of Information from Health Canada

Health Canada issued a review of toxins in blue-green algae (Cyanobacteria) in 2008. 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 (Health Canada, 2008a). 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 nonspirulina Cyanobacteria by children be discontinued until further studies have been conducted (Health Canada, 2008a).

The Canada Vigilance Program (CVP) listed 8 adverse event reports involving Cyanobacteria and associated with the use of spirulina-containing products between 1965 and 30 June 2009 (the most recent update). Table 4 provides details of these 8 case reports. Insufficient details are available regarding the identity of all the ingredients in the polyherbal products or the quality of the products, as well as dose and duration of intake. The reports are confounded by multiple suspect products and concomitant medications, and in some cases patient history is inadequate (CVP, 2009 and personal communications). Thus DSI-EC cannot adequately review these cases for safety.

Australian Adverse Event Reports

In May, 2005, the Australian TGA changed the regulatory status of *S. maxima* and *S. platensis* from "excipient only" to "active ingredient" (TGA, 2005). Further, the TGA Herbal Ingredient Names Committee determined that the correct Australian Approved Names for these two species should be *Arthrospira maxima* and *A. platensis*, respectively, but the committee allows the use of the more frequently used term *spirulina* elsewhere on the label. TGA provided details of 8 case reports related to spirulina use received up to July 2009. Case #173126 reported concurrent use of kava (*Piper methysticum*). Because of earlier AERs associated with kava, this report was excluded from the present analysis. Two other reports (#181731 and #215572) described headache, dehydration, diarrhea, nausea, dysphasia, renal failure, and oropharyngeal pain, from which the individuals

Table 3 Details of MedWatch cases reporting other outcomes

Case Number	Report / Received Date	Pathology	Comments
# 14036	21 March 2003	Severe hypercalcaemia, dehydration, death	N/A[a]
# 14643	07 February 2001	Transient ischemic attack	N/A
# 14926	26 June 2001	Severe diarrhea, vomiting, grand mal seizures, coma	N/A
# 16471	14 January 2003	Severe parkinsonian syndrome[b]	Product label, manufacturer unknown
# 102994	05 May 2008	Upper body turning red; throat swelling	Deemed serious adverse event in view of throat swelling shut. Patient on Avalide and Coreg.
# 103049	06 May 2008	Anaphylactic reaction	Patient was treated in ER.[c]
# 103409	20 May 2008	Allergic reaction	Skin flushing and throat swelling shut. Visited ER, though not admitted. Patient and family had no problem with previous 5 bottles.
# 113558	20 May 2009	Heart palpitations	A 37-year-old woman reported ER visit because of reactions one week after using a multi-ingredient product containing spirulina. Manufacturer reported that the retained sample met all specifications.

[a]N/A = information not available.
[b]Patient recovered after 4 months. Doctors suspect a possible neurotoxin, some contaminant in blue-green algae or citrus (grapefruit) supplement, or some interaction with the products the patient was taking. Heavy metals were ruled out.
[c]ER = emergency room.

Table 4 Details of Canada CVP case reports on spirulina-containing products

Case Number	Received Date	Age/Sex	Pathology	Comments
# 131254	24 June 1996	30/F	Abnormal liver function tests and malaise	A blue-green algae supplement used
# 131255	12 May 2000	None recorded	Hepatic cirrhosis	A blue-green algae supplement use for 10 years was reported
# 146563	07 March 2002	36/F	Edema, rash	Patient reported using a spirulina-based product (Greens Plus) and concomitant medications (Nexium, Synthroid, Atarax)
# 148360	09 April 2002	37/F	Sleep difficulties	Patient was using spirulina-containing products for >6 months. Reportedly recovered without complications.
# 169033	02 April 2004	40/F	Insomnia; nausea, vomiting; hyperalertness	Patient used a product containing 25 mg spirulina for 3 days and reported adverse reactions; concurrent medication—Celexa.
# 225870	27 November 2007	Age unknown/F	Nausea; fatigue; numbness	Patient used a product containing approximately 1400 mg spirulina for 4 days and reported adverse reactions; concurrent medication—Synthroid.
# 316823	21 January 2009	68/M	Biopsy of kidney was abnormal, nephrotoxicity, renal failure	Patient was taking several suspect polyherbal products: Electrolyte Stamina tablets, Greens + Extra Energy powder, Recovery powder, and Vigrx Plus tablets, all daily
# 320245	19 March 2009	34/M	Hepatic enzyme levels were increased	Patient was taking polyherbal Greens + powder and capsules plus stimulant Xenadrine-EFX daily for 4 months

recovered, associated with the use of multi-ingredient products containing spirulina. Table 5 provides details of the 5 remaining case reports. Information about the intake of spirulina was available for one case (#182013): The patient initially took 2 tablets of spirulina daily for 4 weeks (further details unknown) without event, but on a separate occasion experienced malaise, abdominal pain, chest pain, pyrexia, tachycardia, tachypnoea, and vomiting when she took one tablet. The case report did not record any treatment for these conditions. This case possibly could be considered an idiosyncratic reaction.

As with MedWatch reports, the TGA reports involved different products, product information was not available in all the reports, and the spirulina content in these products was not analyzed. Further, a pattern in the pathology is not apparent from these reports.

UK MHRA Reports

As of October, 2009, the British Medicines and Healthcare Products Regulatory Agency (MHRA) did not register any adverse reports involving spirulina (MHRA, 2009).

Uppsala Monitoring Centre Reports

The Uppsala Monitoring Centre (UMC; World Health Organization Collaborating Centre for International Drug Monitoring) co-ordinates individual case reports of suspected adverse drug reactions sent by the national pharmacovigilance centers of 96 participating member countries (as of October 2009). By November 2009, the UMC had received 8 nonduplicate reports of suspected adverse reactions associated with spirulina. All the reports classify the product as "Spirulina spp," except for 1 Swiss report that specifies *S. pratensis*. These reports included two reports from Australia that are cited in Table 5 (#178912 and #222614) and one report from MedWatch cited in Table 2 (#15515). The 5 other reports are summarized in Table 6. A comparison of the UMC reports and those from other agencies (such as MedWatch) show that some national reports do not reach UMC. The UMC caveat statement says: "The information shown is not homogeneous at least with respect to origin or likelihood that the pharmaceutical product caused the adverse reaction. The information does not represent the opinion of the World Health Organization."

Animal Pharmacological Data and Reproductive Toxicity

As was the case with the clinical study reports, most publications using animal models investigated the biological activities of spirulina and did not directly evaluate safety. Although several studies investigated the acute, subchronic, and chronic toxicity, reproductive and developmental toxicity, and genotoxicity of microcystin-LR (of nonspirulina origin) (reviewed in Health

Table 5 Details of Australian TGA case reports on spirulina-containing products

Case Number	Received Date	Age/Sex	Pathology	Comments
# 178912	28 August 2002	37/F	Hemorrhage	Intake, duration, identity not recorded
# 182013	23 January 2003	51/F	Malaise, abdominal pain, chest pain, pyrexia, tachycardia, tachypnoea, and vomiting	Positive rechallenge. Patient took spirulina 2 tablets daily for 4 weeks. No treatment.
# 205473	23 February 2005	33/F	Breast milk discoloration	Product intake, duration, identity not recorded
# 211302	18 July 2005	38/F	Photosensitivity reaction; dry mouth, eye pain, headache	Patient used a multi-ingredient product containing spirulina
# 222614	15 September 2006	50/F	Fatigue, muscular weakness	Patient suspects that spirulina reduced effectiveness of venlafaxine (Effexor-XR)

Table 6 Details of UMC case reports on spirulina-containing products[a]

Country of origin	Patient (age, sex)	Details of spirulina use; indication	Date of onset of reaction	Reported adverse reaction(s)	Outcome	De-/rechallenge information	Comment
S Africa	62, F	Taken orally 3 daily, duration of use n/s[b]; rheumatoid arthritis and other inflammatory polyarthropathies	22 September 2000	Rash	Recovered	Reaction abated on drug withdrawal; no rechallenge	No other medicines stated
S Africa	47, F	Taken orally 3 daily, duration of use n/s; indication n/s	20 August 2003	Diarrhea, cramps	Recovered	Reaction abated on drug withdrawal; reaction recurred on rechallenge	Also taking Eugynon
Switzerland	48, F with previous embolism, thrombosis	Taken orally 12 daily starting 6 days before onset of reaction and continuing; malaise and fatigue	11 March 2005	Prothrombin time shortened; drug (aceno-coumarol) ineffective	Recovered	Spiruliana: dose reduced, effect unknown; rechallenge status unknown Acenocoumarol: dose increased, no further information provided	Also taking acenocoumarol for thrombosis (daily; dose n/s) started 11 days before onset of reaction; suspected interaction
Switzerland	n/s, F	*S. pratensis* taken orally 1 daily, duration of use n/s; indication n/s	September 2008	Abdominal pain	Unknown	*S. pratensis* and chromium withdrawn, outcome unknown; rechallenge status unknown	Also taking chromium orally 0.16 mg daily for NIDDM[c] and MSM 1 daily
Malaysia	15, M	Taken orally, 500 mg daily for 11 days starting 2 days before onset of reaction	2 October 2005	Morbiliform rash; pityriasis rosea	Not recovered	No improvement was observed on drug withdrawal; no rechallenge	No other medicines stated

[a]Caveat statement: "The information shown is not homogeneous at least with respect to origin or likelihood that the pharmaceutical product caused the adverse reaction. The information does not represent the opinion of the World Health Organization."
[b]n/s = not stated.
[c]NIDDM: non–insulin-dependent diabetes mellitus; MSM = methylsulfonylmethane.

Canada, 2008b), similar information is not available for spirulina. A review of the literature describing animal experiments with relevance to safety aspects is presented here.

S. maxima was reported to possess hepatoprotective activity in a rat model (Torres-Duran et al., 1999; 2006). At 5% concentration in feed, spirulina exhibited hepatoprotective activity against induction of fatty liver by carbon tetrachloride. Similar protective effects are reported in rat models in cadmium-induced hepatotoxicity (Karadeniz et al., 2009) and in dibutyl nitrosamine–induced liver toxicity (Ismail et al., 2009).

Administration of spirulina in rat models did not result in any reported adverse effects or organ toxicity (Ismail et al., 2009) and had no effect on pregnancy (Kapoor and Mehta, 1993). The safety of *S. maxima* in reproduction was observed in several studies at 10%–20% feed levels (Chamorro et al., 1996a; 1997; Salazar et al., 1996; 1998). In animal experiments for acute, subchronic, and chronic toxicity, reproduction, mutagenicity, and teratogenicity, spirulina did not cause body or organ toxicity (Chamorro et al., 1996b). Protective activity of spirulina (up to 800 mg/kg/day; 2 weeks) was reported in cyclophosphamide-induced mutagenicity in a mouse model (Chamorro-Cevallos et al., 2008).

Literature is scant with respect to the safety of spirulina consumption in lactation. Kapoor and Mehta (1998) reported that feeding rats with spirulina to provide 22% protein during lactation was without safety concerns. No records were found for spirulina in the Drugs and Lactation Database (LactMed; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?LACT).

Supplement–Drug Interactions

Little information is available about the interactions of spirulina and other medications. Small increases in calcium concentrations have been associated with spirulina use, although it is unclear whether this is due to the effects of spirulina alone. Concurrent use of spirulina and calcium supplements may further increase calcium concentrations (NLM, 2009). Because of the observed immunomodulatory activity of spirulina (Mao et al., 2005; Hayashi et al., 1994;1998), its use potentially could interfere with immunosuppressive therapies such as azathioprine, cyclosporine, corticosteroids, and other medicines that have immunological effects. However, at present there is no evidence to support this hypothesis.

Contaminants—Microcystin, Heavy Metals, and Microbial Organisms

The primary concern with respect to the quality of spirulina is the potential for contamination with other blue-green algae, such as *M. aeruginosa*, that produce toxic microcystins. Microcystins are known to be hepatotoxic and carcinogenic because they inhibit protein phosphatases, PP1 and PP2A, leading to hyperphosphorylation of cellular proteins (WHO, 1999). Approximately 60 congeners of microcystins (cyclic heptapeptides) are known, and the predominant one is microcystin-LR. The lethal dose (LD_{50}) for microcystin-LR following oral administration is approximately 5 mg/kg body weight in mouse and rat models

(WHO, 1999; Fawell et al., 1999). The Health Canada (2008b) review of cyanobacterial toxins suggested that a maximum acceptable concentration (MAC) for microcystin-LR in drinking water is 1.5 ppb (1.5 μg/L).

In a mouse model the no observed adverse effect level (NOAEL) for microcystin-LR was determined to be 40 μg/kg-day in a 13-week oral dosing study (Fawell et al., 1999). Applying a 1000-fold uncertainty factor, the Oregon Health Division (Gilroy et al., 2000) determined the permitted total daily intake (TDI) for microcystins to be 1 μg/g (1 ppm) in spirulina products, assuming consumption of about 2 g/day spirulina by adults. Health Canada has published a comparison of the various methods available (ELISA, protein phosphatase inhibition assay, and LC-MS) to determine microcystin concentration levels (Lawrence et al., 2001).

Because spirulina is either harvested from natural, warm, alkaline waters or is grown under controlled salinity conditions, the need to monitor spirulina product quality parameters was recognized for the following contaminants: microcystins (Gilroy et al., 2000; Lawrence et al., 2001), heavy metals such as mercury, cadmium, lead, and arsenic (Johnson and Shubert, 1986; Sandau et al., 1996), and microbial content (Wu and Pond, 1981). Neurotoxic anatoxin-a and its metabolites reportedly were not isolated from samples of food components containing blue-green algae and spirulina collected in Portugal and from urban centers across Canada in 2005 (Rawn et al., 2007). A review of the human health risk assessment for toxins in cyanobacterial blooms (with an emphasis on microcystins in *A. flos-aquae*) concluded that public standards are important for ensuring the quality of spirulina products (Dietrich et al., 2008).

Following the DSI-EC review, the proposed *USP–NF* monograph for spirulina (*S. platensis* and *S. maxima*) will provide specifications and methods of testing for the following parameters: microscopic description; limit of microcystins (assayed by ELISA; not more than 1 ppm); heavy metals (according to *USP* General Chapter <231>, not more than 10 μg/g); content of protein (not less than 60%); and microbial enumeration (according to *USP* General Chapter *Microbiological Enumeration Tests—Nutritional and Dietary Supplements* <2021>).

Regulatory Status

FDA has not made a determination regarding the generally recognized as safe (GRAS) status of spirulina (*A. platensis*). However, FDA "had no questions" in response to the submission of a GRAS Notification package to the agency in which a manufacturer concluded that spirulina has GRAS status for use as an ingredient in foods such as specialty bars, powdered nutritional drink mixes, and popcorn, and as a condiment in salads and pasta at quantities ranging from 0.5 to 3 g per serving (FDA, 2003). In November 2008, an Indian company reported that an independent panel of experts affirmed that the company's organic spirulina was "self-affirmed GRAS" when used at an acceptable daily intake (ADI) of 20 g per day and that it has received permission to use the "USP Verified" mark from the USP Dietary Supplements Verification Program (Parry, 2008). A self-affirmed GRAS substance is one whose safety has been evaluated in terms of evidence and scientific procedures performed by qualified experts and determined to be safe under the conditions of intended use (FDA Redbook, 2007). Left unanswered is how these GRAS substances differ, if at all, from the spirulina products that have been associated with adverse events. Also unknown is the quality standards of the spirulina products purportedly causing the adverse effects. However, given the current status of the DS AERs (Gardiner et al., 2008), it is unfortunate that only a limited amount of information can be obtained from them.

A search of the Dietary Supplements Labels Database (DSLD, 2009) reveals that *spirulina* is found on the labels of more than 80 dietary supplement products. The majority of these products contain multiple components, and the content of spirulina varies from 2 mg to 1000 mg in various formulations such as powder, tablets, or proprietary blends. The maximum recommended serving size for the powder was 7 g per day.

Although historically spirulina was used as a food or food component, no quality monograph is found in the following resources:

British Pharmacopoeia
European Pharmacopoeia
Health Canada
Pharmacopoeia of the People's Republic of China
World Health Organization monographs
German Commission E monographs
WHO International Pharmacopoeia
Japanese Pharmacopoeia
Food Chemicals Codex
ESCOP monographs
British Herbal Compendium
Joint Expert Committee on Food Additives (JECFA) Compendium of Food Additives
Codex Alimentarius

Because spirulina is legally marketed as a dietary supplement in the US and is available in several countries, the current review shows the need for quality standards.

DISCUSSION

As previously noted, spirulina has a long history of use as a food and as a food component. Review of the data indicates that two diverse populations, Aztecs in Mexico and natives of the Sahara desert in Chad, harvested and utilized the nutritional properties of spirulina as early as the 16th Century (Ciferri, 1983). Today, several dietary supplement and functional food products containing spirulina are available in the US market.

Although historically spirulina has been used as a food component, recent animal experiments led to the exploration

of possible immunomodulatory and antioxidant properties of spirulina products in conventional dosage forms (powder or capsules) or as functional foods (Mao et al., 2005; NBJ, 2009; NLM, 2009), often in combination with other ingredients. Thus, the modern uses of spirulina products as dietary supplements and their accompanying structure–function claims (immune system support, anti-oxidant properties, and anti-inflammatory properties) are not totally in accordance with the historical uses of spirulina as food. While these structure–function claims may be based on experimental observations or empirical evidence, additional insight is needed into the mechanisms of action, short-term and long-term effects, and potential harms profile to determine fully the safety of conventional usage of spirulina products (Man, 2009).

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 and are not available for review. Another tool to measure the safety of a product is postmarketing surveillance, which provides valuable information about the safety profile of an ingredient in the general population, in those consumers with chronic conditions, in vulnerable populations, and in special populations such as pregnant or breast-feeding women, older people, children, and prescription medication users. DSI-EC's safety review found very few such clinical study reports and no organized postmarketing surveillance studies for products containing spirulina. A recent review on the evidence-based application of spirulina in clinical practice calls for fully powered clinical trials to substantiate the purported positive effects of spirulina (Karkos et al., 2008). DSI-EC culled 103 AERs for spirulina from several sources (3 clinical case reports, 79 MedWatch reports, 8 Canada CVP reports, 8 Australian TGA reports, and 5 reports from the Uppsala Monitoring Centre). Of these 103 AERs, DSI-EC reviewed 31 cases that contained minimal information (Tables 2–6). Causal attribution for the AERs was challenging because of the data limitations (Gardiner et al., 2008; Mahady et al., 2008). DSI-EC observed that only a weak causality (possible rating) could be attributed to most AERs according to the WHO causality scale (WHO, 2004) or the Naranjo scale (Naranjo et al., 1981). Only one case report from TGA (#182013) reported rechallenge information, but even that case is likely to be an idiosyncratic reaction because the patient initially took only 2 tablets of spirulina and rechallenge occurred with only 1 tablet (quantity of spirulina per tablet is unknown). The recent report from the American Association of Poison Control Centers (Bronstein et al., 2009) indicates only two cases of "moderate" (and none of "major") outcome for blue-green algae products in 2008.

The strengths and limitations of spontaneous AERs are well documented (Gardiner et al., 2008). They provide important safety information from a large and diverse exposed population, compared to premarketing human clinical studies. The importance of AERs is the ability to detect rare adverse events. For instance, to detect an adverse event with an incidence of 1 in 1000 (with 95% confidence), a clinical trial with 3000 patients is required (Lewis, 1981; Hanhley and Lippman-Hand, 1983; IOM, 2005). Further, as observed in an FDA-commissioned study, the agency estimates that it receives less than 1% of all AERs associated with dietary supplements (Woo, 2007). Thus, the evaluation of the AERs aids in generation of important safety-related hypotheses. The core information of an AER consists of 1) the reporter, 2) the patient, 3) the suspect product, and 4) a narrative report of the adverse event. However, DS AERs often require additional information, including details of the DS product (e.g., dose/amount taken and duration of use, brand name, manufacturer, exact names of ingredients as listed on the product label, and the time between product administration and the reaction) and patient characteristics (e.g., age, sex, concomitant use of other medications such as over-the-counter and DS products, and medical and social history such as smoking and alcohol use). The DSI-EC review and analysis of the AERs were affected by the quality of the available AER information. Because DSI-EC constantly monitors AERs concerning the safety of supplements for which *USP–NF* monographs are developed, the safety classification for spirulina may be reevaluated as new information becomes available.

Recommended intake from several manufacturers of spirulina products and other literature sources indicate that spirulina consumption ranges from approximately 1 to 10 g per day, and consumption of up to about 40 g a day is also not uncommon. For this review, members of the DSI-EC critically read the AERs to evaluate the relationship between the dose and duration of use of spirulina at which AERs were observed and the typical consumption of spirulina (1–10 g per day). Reflecting the limitations of the current DS AER system (OIG, 2001), DSI-EC could not establish this relationship from the available information. Further, only limited relevant information is available from animal studies in this regard. In a mouse model, oral administration of 800 mg/kg bodyweight of spirulina for 2 weeks did not lead to any toxic effects (Chamorro-Cevallos et al., 2008).

The total number of spontaneous AERs received is relatively small, although the impact of the introduction of mandatory reporting of serious adverse events in the US remains to be seen. A total of 16 AERs was filed with FDA's MedWatch since January 2008, the period when serious adverse event reporting for dietary supplements became mandatory (Dietary Supplement and Nonprescription Drug Consumer Protection Act, Public Law 109–462, 120 Stat. 3469). Further, researchers need to assess the impact that current cGMP requirements for dietary supplements have on product quality and reporting of adverse events.

This review also finds a need for further research on the safe use of spirulina in special populations. For example, studies are needed to ascertain the safety in usage of spirulina as a dietary (protein) supplement by nursing women. The current review found no information concerning appropriate use recommendations during lactation (Briggs et al., 2008; Blumenthal et al., 2000; Bradley, 1992; Brinker, 2001; McGuffin et al., 1997; Mills and Bone, 2005). DSI-EC encourages nursing mothers to discuss the use of spirulina with their healthcare

professionals. In accordance with *USP–NF General Notices* relating to the labeling of botanical-containing products, the proposed quality monograph for spirulina intended for use as a dietary supplement will contain the following statement: "If you are pregnant or nursing a baby, seek the advice of a health professional before using this product."

Contamination of spirulina with other Cyanobacteria (such as *M. aeruginosa*) is a matter of serious concern because the contaminating culture may produce hepatotoxic microcystins. Eight cases of hepatotoxicity were associated with use of spirulina products, including 1 case report by Iwasa et al. (1982) (with concurrent simvastatin use), 5 MedWatch case reports (including case #15515 involving significant prior medical history, and case #67512 involving a multi-ingredient product), and 2 case reports from CVP (with a product of unknown identity or quality and no data about intake or patient history). These reports do not make clear if the hepatotoxic microcystins were present as a result of contamination with other Cyanobacteria such as *M. aeruginosa*. While some AERs indicated a rare possibility of liver damage from spirulina, data from some experimental studies in animal models and clinical studies indicated no hepatotoxicity from spirulina (Băiuş and Tănăsescu, 2002; Torres-Duran et al., 1999; 2006).

Review of the toxicology of cyanobacteria suggests that microcystins are not produced by spirulina, in contrast to some other blue-green algal species (Health Canada, 2008a). The use of other non-Spirulina species of Cyanobacteria as blue-green algae, including *A. flos-aquae* and *M. aeruginosa*, which also grow in natural, warm, alkaline waters is a safety concern. Gilroy et al. (2000) and Lawrence et al. (2001) showed that a significant portion of the commercially available spirulina products exceed the limit of microcystins beyond 1 μg/g (1 ppm). The recent opinion from the Senate Commission on Food Safety of the German Research Foundation (Eisenbrand et al., 2008) expressed concerns about the concentrations of microcystins in algal products used as food supplements. Cox et al. (2005) reported isolation of neurotoxic β-*N*-methylamino-*L*-alanine (BMAA) from 77% of the Cyanobacteria tested, but the study did not include spirulina. A leading manufacturer reported that its spirulina supplements did not contain BMAA (NTP, 2008). Considering the risk of cyanobacterial toxins from nonspirulina species in blue-green algae, conforming to quality controls is very important to ensure the safety of spirulina.

DSI-EC DELIBERATIONS AND CONCLUSIONS

DSI-EC reviewed literature relating to the safety and toxicology of spirulina to determine whether spirulina could be admitted into *USP–NF* as a quality monograph. After reviewing the information reported here, DSI-EC unanimously decided that the available evidence does not indicate a serious risk to health or other public health concern that precludes admission of a spirulina quality monograph into the compendium. Accordingly, assignment of Class B (prohibiting monograph development) is considered inappropriate for spirulina. DSI-EC based this decision on the lack of a pattern in pathology, lack of information about the products and product quality, confounding variables involving multi-ingredient formulations, and likelihood that the reported adverse reactions may be due to toxic microcystin contamination. 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.

Considering the range of the data reviewed and because of the limited information available in the AERs, DSI-EC 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 *US–NF* when these dietary ingredients are properly identified, formulated, and used. Based on this determination, USP is developing a quality monograph for spirulina and has verified a spirulina dietary ingredient under its Dietary Ingredient Verification Program. In accordance with USP's continuous revision approach (USP, 2009), DSI-EC also reviews new information as it becomes available in periodic safety revisions.

ACKNOWLEDGMENTS

DSI-EC thanks Stefan Schuber, PhD, ELS, director of scientific reports, at USP for his editorial assistance. We thank Australian TGA for providing us information about case reports.

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