

Assessing Potential Health Risks from Microcystin Toxins in Blue-Green Algae Dietary Supplements

Duncan J. Gilroy,¹ Kenneth W. Kauffman,¹ Ronald A. Hall,¹ Xuan Huang,² and Fun S. Chu²

¹Environmental Services and Consultation Section, Oregon Health Division, Portland, Oregon, USA; ²Department of Food Microbiology and Toxicology, University of Wisconsin-Madison, Madison, Wisconsin, USA

The presence of blue-green algae (BGA) toxins in surface waters used for drinking water sources and recreation is receiving increasing attention around the world as a public health concern. However, potential risks from exposure to these toxins in contaminated health food products that contain BGA have been largely ignored. BGA products are commonly consumed in the United States, Canada, and Europe for their putative beneficial effects, including increased energy and elevated mood. Many of these products contain *Aphanizomenon flos-aquae*, a BGA that is harvested from Upper Klamath Lake (UKL) in southern Oregon, where the growth of a toxic BGA, *Microcystis aeruginosa*, is a regular occurrence. *M. aeruginosa* produces compounds called microcystins, which are potent hepatotoxins and probable tumor promoters. Because *M. aeruginosa* coexists with *A. flos-aquae*, it can be collected inadvertently during the harvesting process, resulting in microcystin contamination of BGA products. In fall 1996, the Oregon Health Division learned that UKL was experiencing an extensive *M. aeruginosa* bloom, and an advisory was issued recommending against water contact. The advisory prompted calls from consumers of BGA products, who expressed concern about possible contamination of these products with microcystins. In response, the Oregon Health Division and the Oregon Department of Agriculture established a regulatory limit of 1 µg/g for microcystins in BGA-containing products and tested BGA products for the presence of microcystins. Microcystins were detected in 85 of 87 samples tested, with 63 samples (72%) containing concentrations > 1 µg/g. HPLC and ELISA tentatively identified microcystin-LR, the most toxic microcystin variant, as the predominant congener. **Key words:** *Aphanizomenon flos-aquae*, blue-green algae, cyanobacteria, dietary supplements, microcystins, *Microcystis aeruginosa*. *Environ Health Perspect* 108:435–439 (2000). [Online 27 March 2000] <http://ehpnet1.niehs.nih.gov/docs/2000/108p435-439gilroy/abstract.html>

The presence of toxic cyanobacteria (blue-green algae) in surface waters used for drinking water sources and recreation is receiving increasing attention worldwide as a potential health concern (1–5). Human illness resulting from exposure to blue-green algal toxins, though less common than poisonings of wild and domestic animals (6–9), has been reported (1,2,10–12). In 1996, 55 hemodialysis patients died in Brazil when the dialysis water became contaminated with toxins from the blue-green alga *Microcystis aeruginosa* (13).

Although potential health risks from exposure to algal toxins in surface waters have been widely recognized, potential risks from exposure through another important route—ingestion of contaminated dietary supplements made from blue-green algae (BGA)—have received only limited attention (2,3,6,14). Because of its concentrated form, the exposure potential to algal toxins in contaminated BGA products can greatly exceed potential exposure through water consumption or recreational contact.

Since 1983, the blue-green alga *Aphanizomenon flos-aquae* has been harvested from Upper Klamath Lake (UKL) in southern Oregon and marketed as a dietary supplement. These supplements are commonly consumed in the United States, Canada, and Europe for their putative beneficial effects,

including “detoxification,” increased energy, elevated mood, and weight loss. They are also marketed for use in children and are used as a treatment for Attention Deficit Hyperactivity Disorder (15).

In September 1996, the Oregon Health Division (OHD) in Portland, Oregon, learned that UKL was experiencing an extensive bloom of *M. aeruginosa*, a blue-green alga that produces potent hepatotoxins called microcystins. An investigation revealed that the *M. aeruginosa* bloom had resulted in significant microcystin levels in the lake, and an advisory was issued recommending against water contact. The advisory prompted calls from consumers of UKL BGA products, who expressed concern about the possible contamination of these products with microcystin toxins. In response to these concerns, the OHD and the Oregon Department of Agriculture (ODA; Salem, OR) tested BGA-containing supplements for microcystins and derived a regulatory standard for microcystins in BGA products.

In this paper we discuss the rationale for Oregon’s microcystin standard and report analytical test results that clearly indicate the need for regulatory control of BGA products.

Materials and Methods

Materials. Microcystin-leucine-arginine variant [microcystin-LR (MCYST-LR)] was

provided by W.W. Carmichael (Wright State University, Dayton, OH). We obtained ELISA-grade horseradish peroxidase (HRP) from Boehringer Mannheim Biochemicals (Indianapolis, IN). We prepared polyclonal antibodies against MCYST-LR used in an ELISA as previously described (16). MCYST-HRP conjugate was prepared according to the method of Chu et al. (16). The antibodies used in the assay were most specific for MCYST-LR (100%) and arginine-arginine variant (MCYST-RR; 126%), with some cross-reactivity with tyrosine-arginine variant (MCYST-YR; 65%) and nodularin (48%) (16). K-B substrate solution (a premixed solution with 1 mM 3,3',5,5'-tetramethylbenzidine and 3 mM H₂O₂ potassium citrate buffer, pH 3.9) was supplied by ELISA Technologies (Lexington, KY). Sep-Pak C-18 reversed-phase cartridges were from Waters Associates (Milford, MA). All other chemicals and organic solvents were of reagent grade or better.

Product testing. In 1996 and 1997, OHD investigators obtained 61 product samples (tablets or capsules) from the four primary UKL BGA harvesters. The samples were obtained from various Oregon retail outlets or directly from the harvesters. Fifteen samples of Spirulina (a commonly consumed BGA that is not from UKL) and 26 additional UKL BGA samples were obtained by ODA investigators in 1998 and 1999.

A competitive direct-ELISA (cd-ELISA) was used for the analysis of MCYST content in BGA samples according to the protocol of Chu et al. (17). Typically, 0.5–1 g of a well-mixed algae sample was homogenized with 50 volumes 0.1 M ammonium bicarbonate in a tissue homogenizer for 3 min. For tablets, two randomly selected samples were first ground with a mortar; 0.5 g of the mixed sample was used. After homogenization and centrifugation (30 min at 16,000 × g) to remove the cell debris or solid materials,

Address correspondence to D.J. Gilroy, Oregon Health Division, 800 NE Oregon Street, Suite 608, Portland, OR 97232 USA. Telephone: (503) 731-4015. Fax: (503) 731-4077. E-mail: duncan.j.gilroy@state.or.us

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we diluted the supernatant 10 and 100 times with distilled water and then subjected it to the cd-ELISA using the protocol previously described by Chu et al. (17). At least two analyses were performed for each sample, and triplicates were run for each analysis. The intrawell coefficient of variation of the same sample extract in the triplicates was generally < 10%. The analytical recovery of MCYST-LR added to the blank algae extract sample (1–20 µg/g) in the ELISA was > 70% (17).

Because of the known heterogeneous distribution of *M. aeruginosa* and microcystin toxin in lake environments (18) and the consequent potential implications for quality assurance of BGA products, we also tested BGA samples for microcystin homogeneity. We evaluated homogeneity in two ways: *a*) by comparing the microcystin content of a single tablet or capsule from a product container with a composite tablet/capsule from the same container; and *b*) by comparing microcystin concentrations in samples from different product containers from the same batch (i.e., those having the same batch number).

To further elaborate on the ELISA data from the BGA samples, two samples were subjected to HPLC separation and ELISA analysis of the collected fractions. We used a Spherclone ODS C-18 reversed-phase column (4.6 mm × 25 cm; Phenomenex, Torrance, CA) and a Beckman model 100 A HPLC system (Beckman, Fullerton, CA) equipped with a 412A controller for the analysis. In a typical experiment, extracts prepared from a 3-g sample (1 g each time with 30 mL of buffer) were pooled, diluted with water (1:10), and subjected to a C-18 reversed-phase Sep-Pak column, as previously described (17). After washing with distilled water and 20% methanol, microcystin was eluted from the column with 3 mL 100% methanol, which was evaporated to dryness and redissolved in 0.2 mL methanol followed by 0.8 mL distilled water. The recovery of MCYST-LR in algal extract after Sep-Pak treatment was generally > 88%, in the range of 1–63 µg/mL (17). For HPLC, 50 µL of this solution was diluted with 50 µL 0.02 M phosphate-buffered saline (PBS) and 10–50

µL (depending on toxin concentration) of the diluted solution was injected into the column. The column was equilibrated with solvent A [CH₃OH:H₂O: TFA (20:80:0.025), vol/vol] at a flow rate of 1 mL/min. Five minutes after the injection of samples, we applied a gradient elution starting from 100% A to 100% B [CH₃OH:H₂O: TFA (90:10:0.025), vol/vol] over a period of 20 min. This was followed by solvent B for another 20 min, and finally by 100% solvent A over a period of 20 min before the next injection. Each fraction (0.5 mL/fraction) collected from the HPLC column was diluted (1:1000) with PBS and then subjected to the ELISA.

Derivation of a maximum allowable concentration for microcystins. We used established risk assessment methodology (19) to develop a tolerable daily intake (TDI) and a maximum allowable concentration for microcystins in BGA products. The TDI was based on a no-observed-adverse-effect level (NOAEL) for microcystin-LR in mice (20) with the application of standard uncertainty factors (UFs) (19). The approach is similar to that used recently by Health Canada (21) and the World Health Organization (22) to develop guideline levels for microcystins in drinking water.

Results

ELISA standard curve. A typical standard curve for ELISA analysis of MCYST-LR is shown in Figure 1. Although the standard curve covered a range from 0.02 to 5.0 ng/mL, data within the linear range of the curve (0.05–1.0 ng/mL) were the most accurate. Initial screening found that 1:10 and 1:100 PBS dilutions were sufficient for most samples to fall within the linear range. All diluted sample solutions were again subjected to ELISA to ensure that the data were within the linear range.

Product testing. Analytical results for microcystins in BGA products collected between 1996 and 1999 are shown in Table 1. The average MCYST levels in the samples collected by OHD investigators from the four primary harvesters in 1996 and 1997 ranged from 2.15 to 10.89 µg/g.

Microcystin concentrations in ODA samples collected in May 1998 were generally similar to OHD sampling results. Lower concentrations were observed in samples collected in January 1999.

We observed differences between individual and composite samples from the same BGA product containers, although in most cases (21 of 29) the variation was < 30% (data not shown). Three samples showed substantial variation (53, 76, and 99%), indicating that microcystin heterogeneity can occur in some BGA products. We observed similar results when we compared microcystin concentrations in different product containers from the same batch (Table 2). Variation of the paired analyses ranged from 13 to 63%. The largest variation (0.3 and 0.78 µg/g) was found in batch sample 4, which had levels close to the present ELISA detection limit. In the other paired samples the variation was < 30%; however, a third sample from one batch (batch 5) showed a markedly lower microcystin concentration than the two other samples (< 0.04 µg/g as compared to 3.02 and 4.41 µg/g), again indicating that microcystin heterogeneity can occur in BGA products.

Two algal samples were subjected to HPLC–ELISA. Results of a representative activity profile of the 0.5 mL fractions are shown in Figure 2. Figure 2B shows a major inverse peak with a retention time of 25 min and a minor inverse peak with a retention time of 21.4 min when 10 µL BGA extract (15 mg BGA) was injected and anti-MCYST-LR antibodies were used in the ELISA. The injection of standard MCYST-LR and MCYST-RR separately into the column resulted in single inverse peaks with retention times of 25 and 22 min, respectively (data not shown), which is consistent with our earlier studies (23). Thus, in terms of activity, we identified the large inverse peak in the ELISA–HPLC chromatogram as MCYST-LR and the minor inverse peak as MCYST-RR. Using the ELISA standard curve, we estimated the MCYST-LR concentration in each fraction; the results are shown in Figure 2A (positive peak). The

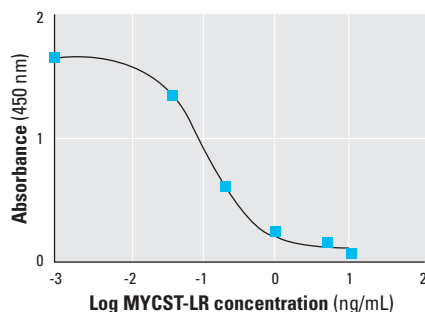


Figure 1. ELISA standard curve for the analysis of microcystin-LR.

Table 1. Microcystin concentrations in UKL blue–green algae dietary supplements.^a

Sample date	Harvester			
	A	B	C	D
1996	10.06 ± 7.42 ^b (2.5–18.4; 8) ^c	5.68 ± 4.83 (0.04–16.36; 14)	2.15 ± 1.81 (0.36–3.76; 4)	2.73 ± 3.32 (0.16–7.84; 6)
1997	10.89 ± 5.37 (0.22–15.2; 6)	6.67 ± 5.64 (0.41–16.44; 10)	2.60 ± 2.89 (0.16–7.96; 8)	4.30 ± 2.24 (1.26–6.79; 5)
1998	16.42 ^d	^e	1.76 ± 2.29 (0.3–6.38; 6)	7.46 ± 2.16 (3.83–9.1; 5)
1999	2.65 ± 2.81 (0.4–6.58; 4)	2.00 ± 1.90 (0–4.14; 5)	0.43 ± 0.44 (0–0.87; 3)	2.38 (0–4.75; 2)

^aSamples were obtained by the OHD (Nov–Dec 1996 and Aug–Dec 1997) and by the ODA (May 1998 and January 1999).

^bValues are means ± SD; microcystin concentration is given in micrograms per gram. ^cValues in parentheses are range and number (n) of samples analyzed. ^dOnly one sample was analyzed. ^eNo sample was analyzed.

total concentration of MCYST-LR in this peak was estimated at 21 ng. Because the antibody has a higher cross-reaction with MCYST-RR (126%) than with -LR (100%), the -RR peak appears to be large; however, the actual amount, estimated as MCYST-LR, was much less (approximately 1.8 ng). Thus, even though MCYST-RR was present, > 90% of the microcystin was the -LR variant. Similar data were obtained for the other sample, which also contained predominantly -LR (24.2 ng) with small amounts of -RR (1.0 ng).

These results are consistent with previous work by others (24) using amino acid analysis and mass spectrometry that showed the predominant microcystins in UKL BGA are MCYST-LR and MCYST-LA. MCYST-LA has very little (< 2%) cross-reactivity with the MCYST-LR antibody (16). Because the toxic potency of MCYST-LA and MCYST-LR is believed to be similar (25,26), it is likely that use of the ELISA for analyzing microcystins in BGA products underestimates the presence of toxic microcystins to some extent.

Mean MCYST-LR concentrations in Spirulina products obtained by ODA investigators in May 1998 and January 1999 were 0.15 and 0.52 µg/g, respectively (Table 3). Although the average microcystin concentrations were low, one sample contained 2.12 µg/g.

Microcystin TDI and regulatory standard. We derived the microcystin TDI by applying three 10-fold UFs to the MCYST-LR NOAEL of 40 µg/kg-day established by

Fawell et al. (20), as follows: a 10-fold factor for extrapolation from animal studies to humans; a 10-fold factor for varying susceptibility in the human population; and a 10-fold factor to account for a) subchronic to chronic extrapolation; b) evidence for tumor promotion; and c) database inadequacies (e.g., the lack of a reproductive study).

Thus, a 1,000-fold total UF was applied to the NOAEL of 40 µg/kg-day to give a TDI of 0.04 µg/kg-day. Applying this result to a 60-kg adult results in 0.04 µg/kg-day × 60 kg = 2.4 µg/day. Assuming a 2-g/day BGA consumption rate (based on product literature and discussions with BGA producers and consumers), 2.4 µg/day ÷ 2 g BGA/day = 1.2 µg/g ≈ 1.0 µg/g. Thus, for adults, a safe level for microcystins in BGA products was determined to be 1 µg/g. This level was adopted by the ODA as a regulatory standard for BGA products on 23 October 1997.

Discussion

Exposure to microcystins and other cyanobacterial toxins through the ingestion of contaminated water or recreational contact is receiving increasing attention around the world as a public health concern (1–5). Several countries (20,21,27), and recently the World Health Organization (22), have made recommendations regarding safe algal toxin levels in drinking water and surface waters used for recreation. In contrast, potential health risks from exposure to toxins in health food products made from BGA have received little attention (2,3,6,14). (Health Canada issued an advisory for microcystins in BGA products on 5 May 1999.) Although the BGA in many health food products is produced under cultured conditions—where the growth of toxin-producing cyanobacteria can, at least theoretically, be controlled—the BGA in products from UKL are harvested from an open environment where the growth of the toxic BGA *M. aeruginosa* is a regular occurrence. Because *M. aeruginosa* coexists with *A. flos-aquae*, the target BGA, it can be collected inadvertently during the

harvesting process, resulting in contamination of BGA products.

In fall 1996, a large bloom of *M. aeruginosa* in UKL prompted the OHD and the ODA to establish a regulatory limit for microcystin toxins in BGA products and to test these products for the presence of microcystin toxins. Using established risk assessment methodology, OHD investigators derived a safe microcystin level of 1 µg/g in BGA products. This level was adopted as a regulatory standard by the ODA on 23 October 1997 for BGA products sold in Oregon. The level is directly comparable to the 1 µg/L drinking water guideline level (based on a daily water intake of 2 L) recently promulgated by the World Health Organization (22). Analysis of UKL BGA products using ELISA found microcystins in 85 of the 87 products tested, with levels ranging up to 16.4 µg/g. Sixty-three samples (72%) had microcystin concentrations > 1 µg/g. HPLC separation and ELISA analysis of selected samples showed that the predominant microcystin (> 90%) was MCYST-LR, which is considered the most toxic microcystin variant.

Because of the known hepatotoxic potency of microcystins, the presence of these toxins in food products is cause for concern. The toxicity of microcystins has been extensively studied (27–31). After ingestion, microcystins are actively taken into the portal circulation from the digestive tract and rapidly cleared from the plasma via active transport into hepatocytes (32–35). Once inside hepatocytes, microcystins tightly bind and inhibit protein phosphatase 1 and 2A (PP1 and PP2A), resulting in the hyperphosphorylation of cellular proteins with critical roles in the maintenance of cell structure and function (31–34,36). Acute exposure to high microcystin doses results in severe damage to the hepatocyte cytoskeleton, causing morphologic changes in hepatocytes and sinusoids and, ultimately, intrahepatic hemorrhage and hypovolemic shock (7,14,28).

Although less is known about the possible health effects from exposure to lower doses of microcystins, there is increasing concern that chronic exposure to low levels may also pose a significant health risk. Potential tumor promotion is a primary concern (14,29,37–39). Microcystins are believed to promote tumor formation through the inhibition of PP1 and PP2A, which are integrally

Table 2. Microcystin concentrations in two UKL blue-green algae samples from each of six batches.

Batch no.	MCYST (µg/g)	Mean	SD	%
1	3.56; 2.69	3.13	0.62	19.81
2	10.42; 6.98	8.70	2.43	27.93
3	3.02; 2.52	2.77	0.35	12.64
4	0.78; 0.30	0.54	0.34	62.96
5	3.02; 4.41 ^a	3.72	0.98	26.34
6	6.02; 7.22	6.62	0.85	12.84

^aThe MCYST concentration in a third sample from this batch, obtained by the ODA in January 1999, was below the detection limit (< 0.04 µg/g).

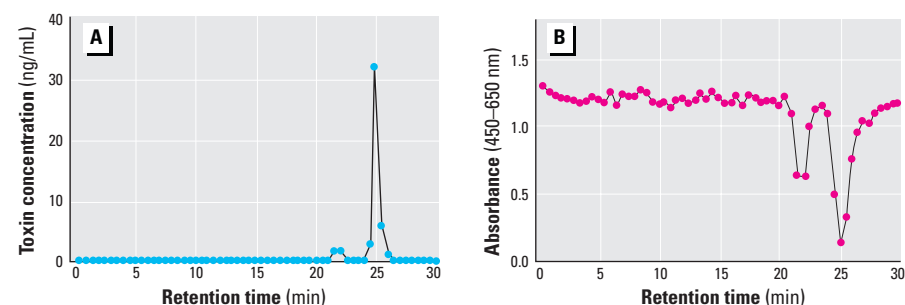


Figure 2. HPLC-ELISA of microcystins in a BGA sample. The flow rate of HPLC was 1.0 mL/min. Fractions (0.5 mL/tube) were collected from the HPLC column and analyzed by ELISA. (A) The actual MCYST concentrations in each fraction, as determined by ELISA. (B) The inverse peak generated by plotting the absorbance readings for each fraction obtained from ELISA against the retention time.

Table 3. Microcystin concentrations in Spirulina blue-green algae dietary supplements.^a

Sample date	MCYST (µg/g)
1998	0.15 ± 0.08 ^b (0.06–0.32; 8) ^c
1999	0.52 ± 0.77 (0.0–2.12; 7)

^aSamples collected by the ODA (May 1998 and January 1999). ^bValues are means ± SD. ^cValues in parentheses are range and number (n) of samples analyzed.

involved in cell-cycle regulation (14,29,39). This mechanism of tumor promotion has been well characterized in the diarrhetic shellfish poison toxin okadaic acid (39,40). Evidence for tumor promotion by microcystins has been provided in animal (30,38,39) and epidemiologic (14,39,41–43) studies. Recent work suggests that the high incidence of primary liver cancer in China may be due, in part, to chronic exposure to microcystins in drinking water (39,41–43).

Continuous low-level exposure to microcystins may also result in hepatic accumulation. Studies have shown that, once taken up by the liver, microcystin excretion occurs very slowly (31,34,35). Bioaccumulation of microcystins has been demonstrated in laboratory animals (31) and in aquatic vertebrates and invertebrates (44). These results raise concerns that long-term exposure to even very low levels of microcystins may be significant, and could ultimately result in liver cancer and other liver diseases.

Although it is widely recognized that exposure to microcystins in drinking water represents a significant health risk, the potential for microcystin exposure may be substantially greater for consumers of BGA products. Because *M. aeruginosa* blooms are seasonal events, microcystin concentrations in surface waters tend to be sporadic, thus allowing for regular clearing of microcystins from the liver and recovery from possible injury. However, BGA for use in dietary supplements is harvested during the bloom season and is then processed and distributed all year. Thus, if the BGA is contaminated with microcystins, the toxin exposure is continuous throughout the year. In addition, the intake of toxins from drinking water is naturally limited by water consumption rates (average 1.5–2 L/day), whereas there is virtually no limit to how much BGA can be consumed. Users of these products have reported consumption rates of as much as 20 g/day. Thus, potential exposure to microcystin toxins in BGA products is much greater than exposure through drinking water.

Concerns regarding the potential microcystin contamination of UKL BGA products also raised concerns about the safety of other BGA products, including those containing *Spirulina*. *Spirulina* is a commonly consumed BGA that is grown under cultured conditions (3). Contamination of UKL BGA products occurs because the BGA is harvested from an open lake environment where other cyanobacteria can grow freely; thus, it would be expected that contamination of *Spirulina* and other cultured BGA would be less likely. The present test results, showing low levels of microcystins in *Spirulina* products, appear to support this conclusion. However, although cultured growing conditions provide the

opportunity for careful monitoring and control of toxic algal growth, there is no guarantee that such monitoring and control actually occurs. Without clear regulatory standards for microcystins and other potential contaminants, the purity of any BGA product cannot be assured.

Some BGA harvesters have reportedly made an effort to reduce the microcystin content of their products. The lower microcystin levels in the ODA January 1999 sampling (Table 1) suggest that the industry may be having some success in this regard. However, environmental conditions at UKL in 1997 and 1998 were less conducive to *M. aeruginosa* growth than conditions in 1996, and it is possible the decreased microcystin levels are due, at least in part, to reduced *M. aeruginosa* growth.

A key element for ensuring the safety of BGA products is adequate product testing and batch characterization. The distribution of *M. aeruginosa* and microcystin toxin in lakes can be markedly variable both temporally and spatially (18). Results in Table 1 clearly indicate a wide range of MCYST in the samples analyzed. It is likely this variation is due largely to seasonal differences. Routine testing has shown that MCYST levels in BGA products varies greatly with the time of harvest (45). For example, BGA harvested during periods of heavy *M. aeruginosa* blooms (July–September) contains the highest levels of toxins, whereas MCYST levels in BGA harvested in the late fall (October–November) is generally very low. In addition to temporal factors, the microcystin content in BGA products may also be influenced by spatial variability. In the present study, a comparison of microcystin concentrations in individual and composite samples from the same BGA product containers found that in most cases the variation was < 30%; however, because preliminary data showed differences within some product samples of up to 99%, we further investigated the potential for batch heterogeneity. A comparison of two samples from each of six different batches found unremarkable differences in most of the samples, but found a large difference (> 100%) in one sample.

Historically, batch designations by the UKL BGA industry have been somewhat arbitrary and have differed significantly among harvesters. In some cases, harvesters have relied on a single microcystin analysis for each batch, assuming batch homogeneity. The present findings suggest that batch homogeneity cannot always be assumed, and underscore the need for adequate batch characterization. Overall, these results indicate that microcystin contamination of BGA products can be variable, and that regular monitoring, extensive testing, and thorough

batch mixing is necessary to ensure the safety of BGA products.

BGA products and other dietary supplements are regulated by the U.S. Food and Drug Administration (FDA) under the 1994 Dietary Supplement Health and Education Act (DSHEA) (46). Under this law, the manufacturers of dietary supplements can market their products without having to demonstrate the product's safety to the FDA (47–49). Once a dietary supplement is marketed, the FDA cannot take action against a product unless it can demonstrate that the product is unsafe (49,50). For information regarding the safety of dietary supplements, the FDA relies heavily on reports to its MedWatch program (51). Although this program provides useful information to the FDA, it has several limitations. It is a passive system, and thus only a small percentage of adverse events are recorded. It is also more useful for identifying short-term overt effects (e.g., cardiac arrest associated with ephedrine-containing products) than long-term effects such as liver cancer and other chronic diseases. It is also likely that many potentially adverse events go unrecognized. For example, one reported effect of low-level exposure to microcystins is gastrointestinal (GI) disturbance (2,3,5), and GI disturbance is apparently a fairly common experience of BGA consumers. MedWatch has received several reports of nausea, vomiting, and diarrhea associated with BGA consumption (51). Although GI disturbance is acknowledged by the BGA industry as a potential consequence of BGA consumption, it is attributed to "detoxification" of the body.

Many consumers of dietary supplements and other alternative health care products assume that these products could not be sold without the absolute assurance of safety. Unfortunately, because of the regulatory limits on the FDA that are imposed by the DSHEA, this is not the case. As our work with UKL BGA demonstrates, the dietary supplement industry is largely self-regulated, and assuming that these products are entirely safe may not, in fact, be a safe assumption.

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