CYANOBACTERIAL TOXINS IMPACT CROPS AND ACCUMULATE TO LEVELS CONSIDERED UNSAFE FOR CONSUMPTION.

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Oregon surface waters are experiencing increased occurrence of potentially toxic cyanobacterial blooms (Fig. 1). It is necessary to use the modifier "potentially" because there is no toxin monitoring in the state of Oregon. Advisories are currently based on cell counts of potentially harmful cyanobacteria. This summary is intended to briefly review the recent research on the effects of a cyanobacterial (a.k.a. Blue-green algae) toxin, microcystin-LR on crop plants and highlight two human exposure routes important to Oregon consumers and growers. The toxin microcysyin-LR is one of many toxins (there are 70 analogs of microcystin alone) produced by cyanobacteria. Much of the research focuses on this compound because it is widespread, produced by many species of cyanobacteria and is hydrophobic. This may allow microcystin-LR to diffuse across cell membranes but the exact mechanism of uptake is not known.

An extensive literature review [1] indicates that plants are generally not killed by realistic levels of cyanotoxins but, rather are growth inhibited, which lowers crop yields and unfortunately requires that we consider the possibility of human exposure via the consumption exposed plants.

Microcystin-LR is a potent inhibitor of phosphatases.

Microcystin is a potent inhibitor of protein phosphatases 1 and 2A in both animals and plants [2]. Protein phosphatases are key regulatory enzymes in catalyzing dephosphorylation of serine/threonine residues in phosphoproteins. In humans, microcystins mainly impact the cytoskeletal phosphatases in liver tissue. Disruption of cytoskelatal phosphorylation leads to swelling of cells, hemorrhaging of the liver and possibly death [3]. In plants, protein phosphatases regulate important cellular processes such as ion channel activity, carbon and nitrogen metabolism, tissue development and photosynthesis. It has been shown that plant seedlings can take up microcystin [4, 5], causing inhibitory effects on development, root growth and photosynthesis [6-9]. Necrotic lesions on leaves are also observed and likely due to microcystin induced oxidative stress [6].

Microcystin-LR causes oxidative stress

Plant exposure to microcystin results in the generation of reactive oxygen species such as hydrogen peroxide. If the antioxidant capacity of the plant is overwhelmed then radical oxygen can lead to cell death through necrosis or programmed cell death pathways. The accumulation of hydrogen peroxide can lead to peroxidation of lipids, which are found to accumulate in microcystin exposed plants [5]. Accumulation of peroxide species induces a general xenobiotic decontamination pathway present in both plants and animals [10]. This pathway involves the binding of glutathione to the toxin, resulting in a less- or even non-toxic conjugate that is transported out of the cytoplasm. However, accumulation of toxin in plant tissues demonstrates that this pathway can be overwhelmed.

Plants accumulate microcystin-LR

To date, 15 common crops have been examined for accumulation of microcystin-LR. These studies have found that all plants examined were able to grow in the presence of environmentally realistic levels of microcystin-LR and accumulate toxin. Peuthert et al. placed 6 day old plants in medium containing either purified microcystin or cell free crude extracts of cyanobacterial laden lake water [5]. The 11 crop plants examined accumulated both toxin and peroxidated lipids in shoots and roots to high enough levels that consumption of even a small amount of plant tissue would exceed the WHO consumption recommendation (Table 1). In another study, Crush et al. examined four mature (45-72 days old) crops grown in sand culture [4]. The plants were irrigated either at the base of the plant or over the shoots with water containing cyanobacterial cells. Water applied to the shoots ran off of the rape and ryegrass, with very little wetting of the foliage, and absorption of microcystin by the shoots was not observed. Both lettuce and clover leaves were visibly wetted and retained microcystin. This suggests that leaf cuticle properties are very important in controlling absorption of microcystin applied to plant shoots. This study also found no evidence of translocation of microcystin from roots to shoots [4], although others have observed translocation [5].

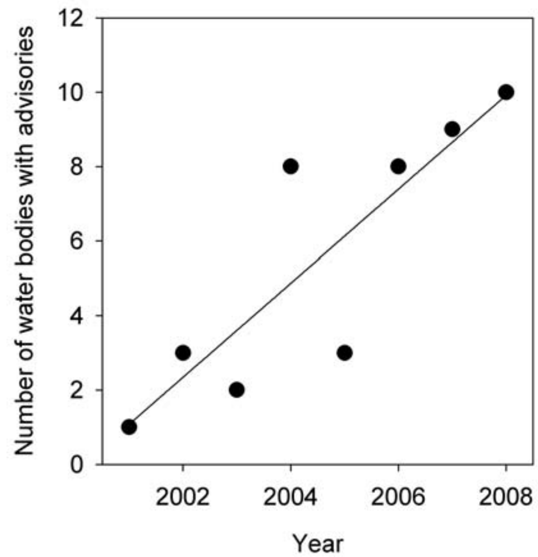

Figure 1. The number of advisories issued by the Oregon Department of Health Services since 2001, the year the program began. This trend is likely due to both the increase in cyanobacterial bloom frequency as well as increased awareness in Oregon. Water bodies with multiple advisories within a season were counted once. Current as of Oct. 24, 2008.

Human health is at risk

Crop plants exposed to environmentally realistic concentrations accumulate microcystin-LR to levels where even a small amount of plant material (0.7 - 9 ounces) would exceed the WHO recommended consumption limit (Table 1). This consumption limit is based on acute toxicity in pigs [11] and does not consider the potential carcinogenicity of microcystin [12]. In addition, overhead irrigation of crops with water containing cyanotoxins posses an inhalation risk to field workers. Exposure symptoms include facial rashes, asthmatic signs and dry sporadic cough with vomiting on the days of, and after, exposure [13].

Table 1. Accumulation of microcystin in 12 crops and the calculated consumption limit assuming a 60 kg (132 lbs) person and using the WHO daily consumption limit of 40ng/kg. I also assume that the fruits will have the same concentration as the shoot. Fruits have not been analyzed by any study to date. All shoot load data from [5], except lettuce [4].

Crop	Shoot load (ng toxin/g w.wt)	Consumption Limit (g)	Consumption Limit (oz.)
Green Pea	27	88.9	3.1
Sugar Pea	28	85.7	3.0
Chick Pea	12	200.0	7.1
Green Mung bean	30	80.0	2.8
Red Mung Bean	30	80.0	2.8
French Bean	40	60.0	2.1
Soya Bean	10	240.0	8.5
Alfalfa	125	19.2	0.7
Lentil	12	200.0	7.1
Maize	37	64.9	2.3
Wheat	125	19.2	0.7
Lettuce	79	30.4	1.1

References Cited

1. Babica, P., L. Blaha, and B. Marsalek, *Exploring the natural role of microcystins - A review of effects on photoautotrophic organisms.* Journal of Phycology, 2006. **42**(1): p. 9-20.
2. Mackintosh, C., et al., *Cyanobacterial Microcystin-Lr Is a Potent and Specific Inhibitor of Protein Phosphatase-1 and Phosphatase-2a from Both Mammals and Higher-Plants.* Febs Letters, 1990. **264**(2): p. 187-192.
3. Falconer, I.R. and D.S.K. Yeung, *Cytoskeletal Changes in Hepatocytes Induced by Microcystis Toxins and Their Relation to Hyperphosphorylation of Cell-Proteins.* Chemico-Biological Interactions, 1992. **81**(1-2): p. 181-196.
4. Crush, J.R., et al., *Effect of irrigation with lake water containing microcystins on microcystin content and growth of ryegrass, clover, rape, and lettuce.* Environmental Toxicology, 2008. **23**(2): p. 246-252.
5. Peuthert, A., S. Chakrabarti, and S. Pflugmacher, *Uptake of Microcystins-LR and -LF (Cyanobacterial toxins) in seedlings of several important agricultural plant species and the correlation with cellular damage (Lipid peroxidation).* Environmental Toxicology, 2007. **22**(4): p. 436-442.
6. Abe, T., et al., *Microcystin-LR inhibits photosynthesis of Phaseolus vulgaris primary leaves: Implications for current spray irrigation practice.* New Phytologist, 1996. **133**(4): p. 651-658.
7. McElhiney, J., L.A. Lawton, and C. Leifert, *Investigations into the inhibitory effects of microcystins on plant growth, and the toxicity of plant tissues following exposure.* Toxicon, 2001. **39**(9): p. 1411-1420.
8. Pflugmacher, S., et al., *Effects of cyanobacterial toxins and cyanobacterial cell-free crude extract on germination of alfalfa (Medicago sativa) and induction of oxidative stress.* Environmental Toxicology and Chemistry, 2006. **25**(9): p. 2381-2387.
9. Smith, R.D., et al., *Protein-Phosphatase Inhibitors Block Root Hair-Growth and Alter Cortical Cell-Shape of Arabidopsis Roots.* Planta, 1994. **194**(4): p. 516-524.
10. Pflugmacher, S., et al., *Identification of an enzymatically formed glutathione conjugate of the cyanobacterial hepatotoxin microcystin-LR: the first step of detoxication.* Biochimica Et Biophysica Acta-General Subjects, 1998. **1425**(3): p. 527-533.
11. Chorus, I. and J. Bartram, *Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management.* 1999, London: E & FN Spon. 416.
12. Grosse, Y., et al., *Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins.* Lancet Oncology, 2006. **7**(8): p. 628-629.
13. Codd, G.A., et al., *Cyanobacterial toxins, exposure routes and human health.* European Journal of Phycology, 1999. **34**(4): p. 405-415.