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TO: Gerald W. Bowes, Ph.D.
Manager, Cal/EPA Scientific Peer Review Program
Office of Research, Planning and Performance

FROM: Dominic Gregorio, Ocean Unit Chief
DIVISION OF WATER QUALITY

DATE: July 9, 2010

SUBJECT: REQUEST FOR EXTERNAL PEER REVIEW OF CYANTOXIN TOXICITY CRITERIA AND HEALTH-BASED WATER CONCENTRATIONS TO PROTECT HUMAN SWIMMERS, DOGS AND CATTLE

The Division of Water Quality requests, by transmittal of this memorandum, that an external peer review be initiated for the report "Toxicological Summary And Suggested Action Levels To Reduce Potential Adverse Health Effects Of Six Cyanotoxins" produced by the Office of Environmental Health Hazard Assessment (OEHHA) under contract to the State Water Board, per the requirements of Health and Safety Code Section 57004.

Summer cyanobacterial blooms cause concern for local health official in several locations in California. The Klamath River has drawn the most attention, but blooms have been reported in many other locations in California.

Cyanobacteria and associated cyanotoxins affect beneficial uses and therefore are a concern for Regional Water Quality Control Boards and local public health officials in several locations in California. Although USEPA has placed cyanobacteria and the cyanotoxins they can produce on its list of official "candidate contaminants" for which it will eventually develop drinking water regulatory limits called "maximum contaminant levels" (MCLs) under the federal Safe Drinking Water Act, it does not appear this will happen in the near future. In addition, the California Department of Public Health has not independently developed cyanotoxin MCLs. The State Water Board lacks authority to adopt MCLs, per se, but contracted with OEHHA to develop exposure assessment-derived information in order to assist Regional Water Boards, local public health agencies, and other stakeholders (such as the CA Department of Fish and Game) with benchmarks for assessing potential risks to human health and also ecological risks based on reviews of the current scientific literature.

The report will very likely be used for regulatory actions such as for CWA Section 303d listings, Total Maximum Daily Loads, and potentially conditions in NPDES Permits or Waste Discharge Requirements. It is important that the report be peer reviewed while the project team at OEHHA is available, so that it may be finalized and available for eventual regulatory use.

In the report, proposed reference doses (RfD)[1] were developed for six cyanotoxins for humans, dogs and cattle. These RfD were used in a variety of exposure scenarios to estimate maximum concentration levels. The present request is for the review both the RfDs and the exposure assumptions.

We believe that the desirable areas of expertise for peer reviewers of this assessment should be the following, in order of importance:

1. Toxicity Assessment – Dose Response
2. Exposure Assessment – Estimation of Intake by People and Animals

Much of the interest in cyanotoxins is on how and when cyanobacteria produce the toxins. These questions on cyanobacteria biology are beyond the scope of this project. We have treated the cyanotoxins as we would any chemical water contaminant. Therefore, expertise on cyanobacteria without experience in toxicology and exposure assessment would not be sufficient to review this document.

There are four attachments to this memorandum. Attachment 1 summarizes development of the toxicity and exposure assessment, and the proposed Action Levels. Attachment 2 identifies the scientific issues to be evaluated by external the peer-reviewers. Attachment 3 identifies the people who worked on the document. Attachment 4 provides an overview of the natural history of cyanobacteria and the aquatic toxicity of cyanotoxins.

If you have any questions, please contact me at (916) 341-5488 or at dgregorio@waterboards.ca.gov . The contact manager for this project is Kim Ward who can be reached at (916) 341-5586 or at kward@waterboards.ca.gov . The OEHHA staff contact for this proposal is Dr. Ned Butler, who can be reached at 916-323-2865 or at nbutler@oehha.ca.gov. Thank you for your consideration of this request.

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[1] RfDs are doses (expressed in units of mg/kg-day) at or below which adverse health effects are not likely to occur. A central assumption is that a threshold exists below which adverse effects will not occur in a population; however, such a threshold is not observable and can only be estimated. An RfD is a quantitative estimate of the lowest dose at which a toxic effect will occur, combined with uncertainty factors that account for variability in sensitivity in the human population and uncertainty in the toxicity database.

Attachments (4)
cc: Shakoora Azimi Gaylon, DWQ
Dave Siegel, OEHHA
Ned Butler, OEHHA
George V. Alexeeff, OEHHA
John Ferderer, OEHHA

Attachment 1

Plain English Summary of the Cyanotoxin Exposure and Toxicity

Background

Blooms of cyanobacteria have caused concern in California surface water bodies. Some species of cyanobacteria produce potent cyanotoxins. Consumption of cyanotoxin-contaminated water has killed dogs and cattle and sickened people though not necessarily in California. Local health officials have no guidance in addressing cyanobacteria blooms. The State Water Resources Control Board contracted with OEHHA to create health-based criteria to protect people during recreational use of surface water bodies and protect dogs and livestock. The report "Toxicological Summary And Suggested Action Levels To Reduce Potential Adverse Health Effects Of Six Cyanotoxins" describes the basis for these criteria. Local health officials are the target audience for these Action Levels.

Rationale

OEHHA computes each Action Level for the six cyanotoxins from a toxicity assessment and an exposure assessment. The toxicity assessment and the exposure assessment are independent from one another.

- *Toxicity Assessments*: Determine the maximum dose to which a person, dog or cow can be exposed daily without adverse health effects. That dose is called a Reference Dose (RfD).
- *Recreational Exposure Assessment*: Determine the relationship between water concentration and daily dose for people using the water recreationally also estimate the amount of recreationally caught fish consumed by people.
- *Animal Exposure Assessments*: Determine the relationship between water concentration and daily dose for dogs and cattle.
- *Compute Action Levels*: Using the RfDs and the exposure assessment dose/concentration relationships determine the maximum concentrations of the cyanotoxins in surface water that do not pose a health threat to people and domestic animals.

Use of Action Levels

The Action Levels for the six chemicals will be available for use by county and city public health officials. These officials make decisions to close or post water bodies. The RWQCBs may use these criteria to determine total maximum daily loads (TMDLs) for these chemicals. TMDLs have California State regulatory authority in contrast to their use by local health officials.

Attachment 2

Description of Scientific Issues to be Addressed by Peer Reviewers

The statute mandate for external scientific peer review (Health and Safety Code section 57004) states that the reviewer's responsibility is to determine whether the scientific portion of the proposed rule is based upon sound scientific knowledge, judgment, methods and practices. We request that each reviewer's responsibility is to make this determination for each of the following issues that constitute the scientific basis of the of the action levels. An explanatory statement is provided for each issue to focus the review. For those work products which are not proposed rules, as is the case here, reviewers must measure the quality of the product with respect to the same exacting standard as if it was subject to Health and Safety Code Section 57004.

While developing the cyanotoxin action levels, staff identified a number of key issues in the analysis. These are issues on which staff would especially like to have review and receive comments.

General Approach

1. Determination to limit scope of assessment on cyanotoxins. OEHHA limited the scope of the assessment to establishing water concentration levels for four forms of microcystins, anatoxin-a, and cylindrospermopsin. There was not enough literature on the toxicity of the other forms of microcystin. OEHHA only used exposure to cyanotoxin as the basis for action level development. While others have used cyanobacteria cell count as the basis for the action levels, the correlation between cell count and toxin level is not consistent. Justification is provided.

Toxicity Criteria for the six chemicals

2. Carcinogenesis was not used as the basis for action level development. When fed to rodents after exposure to a genotoxic liver carcinogen, microcystin-LR increased pre-neoplastic lesions in the liver. Microcystins are liver tumor promoters, but there are no lifetime bioassays for microcystins. Because of microcystins' ability to induce liver apoptosis, they have been used as biochemical tools to elucidate the mechanism of apoptosis. OEHHA did not base the microcystin toxicity on liver carcinogenesis because of the lack of an adequate study. The criterion is based on liver toxicity.

3. Studies on cyanobacteria extracts instead of purified toxin were use as the basis for some action levels. Most poisonings reported in domestic animals have led to acute lethality. Given the lack of data on acute toxicity of purified toxins, studies using extracts of cyanobacteria, with quantified amounts of toxin, were

used to develop acute reference doses (RfDs) for microcystin and cylindrospermopsin in domestic animals.

Exposure Assessment

4. Assumptions were made on microcystin air concentrations based on limited information. OEHHA determined that the information for estimating air concentration inhaled by water-skiers or jet boaters was inadequate for establishing a health-based concentration in water. However, OEHHA used the limited information for predicting an air concentration. Justification is provided.

5. Professional judgment was used in estimating cyanotoxin exposure to dogs. In contrast to the drinking water pathway, no information was available regarding the potential ingestion of water and cyanobacteria due to grooming or swimming in dogs (behaviors described prior to reported deaths). The exposure due to grooming was set as a 2mm layer of water surrounding the surface area of a 20kg dog, which is 1.5 L bloom water/ kg body weight. Considering the large intake estimated for coat cleaning, no exposure was estimated for the possibility of gulping water while swimming.

Microcystin Ecotoxicology

6. No action level for fish was considered because of the large additional effort required. Appendix VII describes a preliminary investigation of the available data on the effects of microcystin on fish. OEHHA concludes that there are sufficient data to develop a reference dose of microcystin to protect fish, but that a significant effort would be required.

Reviewers are not limited to addressing only the specific issues presented above, and are asked to contemplate the broader perspective.

(a) In reading the analysis and the development of human and animal action levels, are there any additional scientific issues that are part of the scientific basis of the human and animal action levels not described above? If so, please provide further comments.

(b) Taken as a whole, are the human and animal action levels based upon sound scientific knowledge, methods, and practices?

The preceding guidance is to ensure that reviewers have an opportunity to comment on all aspects of the scientific basis of the human and animal action levels. At the same time, reviewers should also recognize that the OEHHA has a legal obligation to consider and respond to all feedback on the scientific portions of the document. Because of this obligation, reviewers are encouraged to focus feedback on the scientific issues that are relevant to the central elements being proposed.

Attachment 3

List of Study Participants

Office of Environmental Health Hazard Assessment, Project Team and Management

Dr. Ned Butler
Dr. Regina Linville
Dr. Jim Carlisle
Dr. Dave Siegel

State Water Resources Control Board Staff, Contract Management

Ms. Kim Ward
Mr. Dominic Gregorio

Attachment 4

<u>Natural History of Cyanobacteria</u>

Toxin-producing eukaryotic and prokaryotic algae occur in the divisions Chrysophyta (class Prymnesiophyceae), Pyrrhophyta (class Dinophyceae or dinoflagellates), and Cyanophyta (also called "blue-green algae"), with the latter group being most responsible for toxicity-caused disease and death.

Cyanobacteria have a long evolutionary history, with their first occurrence dating back at least 2.7 billion years ago. Cyanobacteria often dominated the oceans after past mass extinction events. They evolved under anoxic conditions and are well adapted to environmental stress including exposure to UV, high solar radiation and temperatures, scarce and abundant nutrients. These environmental conditions favor the dominance of cyanobacteria in many aquatic habitats, from freshwater to marine ecosystems.

Cyanobacteria are anaerobic prokaryotic phototrophs which use sunlight as an energy source via photosynthesis in thylakoids embedded in their cell membranes, since they lack cell organelles such as chloroplasts for this purpose. These cosmopolitan photosynthetic bacteria occupy a diverse range of aquatic and terrestrial environments, and some occur as endosymbionts within eukaryotic organisms such as cycads. Some taxa also have the ability to fix nitrogen from the atmosphere and thus play an important role in the biogeochemical cycling of nitrogen: although freshwater nitrogen-fixers typically exhibit some degree of cell specialization by developing specialized heterocysts for nitrogen fixation, some marine genera (e.g., *Trichodesmium*) conduct nitrogen fixation in the presence of molecular oxygen produced from photosynthesis via the Mehler reaction. Some aquatic genera are colonial (e.g., Anabaena) and typically form filaments, whereas others occur as single cells even during a "bloom" event. Some common taxa, however, be found as dispersed single cells or as macroscopic aggregations (e.g., *Microcystis*). Some form benthic mats within the photic zone of the water column (e.g., *Phormidium*), while others (e.g., *Microsystis*) may form macroscopic "blooms" on the surface of the water column or at various depths within the photic zone.

Cyanobacteria contribute significantly to primary productivity in terrestrial and aquatic food webs as photosynthesizers, and thus also contribute to the production of atmospheric (molecular) oxygen, and are important participants in the biogeochemical cycling of nitrogen and carbon. The tiny marine cyanobacterium *Prochlorococcus* was discovered in 1986 and accounts for more than half of the photosynthesis of the open ocean. Many cyanobacteria even display the circadian rhythms that were once thought to exist only in eukaryotic cells

Cyanobacteria often favor warm water temperatures and high light environments. In a recent study designed to understand the invasive behavior of the toxic bloom forming cyanobacterium *Cylindrospermopsis raciborskii,* for example, the growth of 10 strains of this cyanobacterium were studied under different light intensities and temperatures. All 10 strains grew under a broad range of temperatures and light intensities, suggesting that the invasion of *C. raciborskii* into freshwater ecosystems at mid-latitudes may result from its ability to tolerate a variety of environmental conditions. Regional and global warming could provide this species with better environmental conditions for optimal growth, which occurs at temperatures approximately 30 degrees Celsius.

Dense macroscopic aggregations – often referred-to as "blooms" – may occur seasonally in aquatic environments in temperate regions, or form continuous blooms in subtropical and tropical waterbodies (e.g., in reservoirs, lakes, estuaries, and/or coastal areas in Florida, Maryland, Nebraska, Kansas, California, New York, Canada, Brazil, China, Australia, New Zealand, Japan, Europe, and Africa). Toxin-producing blooms can occur in surface waterbodies with salinities ranging from freshwater (e.g., blooms in the Great Lakes of North America) to brackish (e.g., the recurrent blooms in the Baltic Sea), to hypersaline waterbodies (e.g., the Salton Sea in California). Although one or more genera may predominate in these blooms, most seem to be comprised of several taxa to varying degrees, and blooms commonly include eukaryotic algae such as Cladophera (the latter commonly occurs in freshwater bodies in North America, including California).

Some aquatic taxa form highly resistant spore-like cells ("akinetes") in response to various environmental stressors and may remain in a hypometabolic state for long periods of time. Other taxa (e.g., *Microcystis*) may not form akinetes, but may adapt to adverse environmental conditions through reducing their metabolic rates seasonally in response to factors such as reduced photoperiod, cooler ambient temperatures, and so on, and thereby reduce cyanotoxin production proportionately.

Cyanobacteria include unicellular and colonial genera, and some exhibit both kinds of morphology (e.g., *Microcystis*). Colonies may form filaments (e.g., *Anabaena*), benthic mats (e.g. *Phormidium*) or even hollow balls (e.g., *Microcystis*). Some filamentous colonies show the ability to differentiate into several different cell types: vegetative cells, the normal, photosynthetic cells that are formed under favorable growing conditions; akinetes, the climate-resistant spores that may form when environmental conditions become adverse; and thick-walled heterocysts, which contain the enzyme nitrogenase, vital for nitrogen fixation. Heterocysts may also form under the appropriate environmental conditions (anoxic) when other nitrogen sources become growth-limiting in a waterbody.

Although cyanobacteria are generally regarded as non-motile, the common freshwater strains of *Oscillatoria/Planktothrix* exhibit an "oscillating" motion that is not well-understood at present. Some exhibit a gliding motion which allows for slow movement across some substrates. Many aquatic taxa form gas vacuoles which enable them to move up and down the water column as gases accumulate (floating) or are released (downward movement): most genera possess a range of photosynthetic pigments which enable optimal utilization of reduced light intensities that are often associated with polluted/otherwise turbid waterbodies, and the relative proportions of these pigments may be adjusted with an accompanying macroscopic color change ("chromatic adaptation"). In the absence of a strong current, gas vacuoles enable cyanobacteria to maintain and adjust their positions in the photic zone for maximum efficiency in light capture for optimal rates of photosynthesis.

Many cyanobacteria also form motile filaments, called homogonia, that travel away from the main biomass to bud and form new colonies elsewhere. The cells in a hormogonium are often thinner than in the vegetative state, and the cells on either end of the motile chain may be tapered. In order to break away from the parent colony, a hormogonium often must tear apart a weaker cell in a filament, called a necridium.

When present in the water column as free-floating cells, filaments, etc., cyanobacteria are subject to being dispersed and/or aggregated by winds, currents, tidal action, and so on. A common macroscopic result is the formation and movement of surface "blooms" into shallow areas near shorelines driven by prevailing winds, tides, and/or downstream transport in rivers and streams.

The relative amounts and chemical composition of nutrient sources –especially nitrogen and phosphorus-containing compounds – available in various freshwater and nearshore coastal waters are known to influence cyanobacterial bloom formation and dynamics. Some climatic effects of global change, including variation in rainfall patterns, floods, droughts, dust storms), tropical storms, and intensity of hurricanes can synergistically (along with nutrients) impact cyanobacterial and eukaryotic algal communities and bloom dynamics. The consequences of these changing patterns, their impact on nutrient entry and utilization in the freshwater-marine continuum, and the interactions between nutrients and other environmental factors on cyanobacterial bloom formation and cyanotoxin production have largely not been investigated.

Lake Tanganyika provides an example of several of these ecological and climatic processes at work in influencing cyanobacterial bloom occurrences. Lake Tanganyika, which is a large rift valley lake in east Africa, has been surveyed and records of its nutrients and temperatures have been published several times in the past century. Temperatures have increased in the past century by 0.9 degrees Celsius at 100 meters, with 50% of the heat gained by the lake in the upper 330 meters. The lake records show a century-long warming trend, and impacts on its planktonic ecosystem have been documented. A sharpened density gradient, a consequence of surface warming, has slowed vertical mixing and reduced primary production. The phytoplankton biomass in 2000 was lower than in 1975 by 70%, and the composition of the phytoplankton also changed. Cyanobacteria comprised a larger portion of the total biomass in March to April 2001 than they did during the same time in 1975.

Cyanotoxins as Aquatic Toxicants

Cyanobacterial blooms occur worldwide in fresh, brackish, and marine waters. Cyanobacteria taxa are generally cosmopolitan, and are commonly the dominant phytoplankton group in eutrophic freshwater bodies. Cyanobacterial growth in surface waters is favored by warm temperatures and abundant nutrients.

Both the production rate and toxicity of cyanobacterial toxins are affected by environmental factors such as light intensity, pH, temperature, and nutrient availability. Cyanotoxins are produced and contained within the bacterial cell structure, and small quantities of toxins may be slowly released until the individual cells age and eventually lyse. The release of cyanotoxins by cell lysis can be induced by environmental stress, including ultraviolet irradiation, physical injury, or changes in water chemistry such as increased salinity. Cyanobacterial viruses (cyanophages) living in sediments that cause cell lysis of toxin-producing cyanobacteria: cyanophages – which are abundant in aquatic habitats – are involved in horizontal gene transfer among related taxa. Some cyanophages transmit portions of the genes involved in the production of cyanotoxins, while other cyanophages cause deletion of toxin-related genes from their host's nucleosomes .

Toxins produced by cyanobacteria have resulted directly in the death of a wide range of organisms, including invertebrates, fish, birds, cattle, sheep, dogs, monkeys, and rhinoceros, and other mammals, vertebrates, and invertebrates. Cyanotoxins in drinking water have caused human deaths, and recreational exposures to toxin-containing waters have caused a range of human illnesses, including acute pneumonia, hepatoenteritis, papulovesicular dermatitis (swimmers' itch), and gastroenteritis.

Toxicity in cyanobacteria has been reported since the late 19th century, mostly from poisonings in freshwater environments. These reports describe sickness and death of livestock, pets and wildlife following ingestion of water containing blooms of toxic algae.

The cyanotoxins identified to date include hepatotoxic peptides (e.g., the microcystins and nodularins), a cytotoxic alkaloid, neurotoxic alkaloids (including anatoxin-a(S), which is a naturally-occurring organophosphate pesticide), various saxitoxins, allergens and lipopolysaccharides are also produced. Laboratory experiments have demonstrated that toxins produced by cyanobacteria can be potent inhibitors of protein phosphatases higher plants as well as mammals.

Over 40 strains in 20 or more cyanobacterial genera can produce a range of structurally and functionally diverse cyanotoxins. Freshwater and marine cyanobacteria, encompassing both planktonic and benthic forms, can produce potent toxins, some of which have been well characterized in terms of their toxic effects and some of which are less well-understood. The known cyanotoxins can exert their toxic effects through a variety of mechanisms (depending on the particular toxin): some are potent neurotoxins that can cause respiratory dysfunction and death through paralysis of respiratory muscles, e.g. saxitoxins, anatoxin-a, anatoxin-a(S). Some affect the liver and other organs, e.g. microcystins, nodularin, cylindrospermopsin, and some exert acute irritant effects on the skin and mucous membranes, e.g. debromoaplysiatoxin, lyngbyatoxin A. Some cyanotoxins are potent tumor promoters, e.g. debromoaplysiatoxin, lyngbyatoxin A, microcystins, and nodularin. Long-term, low-dose exposures to microcystins and/or cylindrospermopsin in drinking water are suspected risk factors for the development of some types of cancer. Cyanobacteria are also rich sources of novel bioactive compounds, most of which have not been evaluated with respect to potential toxicity..

Some researchers have suggested that selective browsing by herbivorous fishes on macroalgae removed potential competitors and favored the establishment of unpalatable benthic cyanobacteria in tropical marine environments. Crude extracts and isolated secondary metabolites of several benthic marine cyanobacteria such as *Lyngbya* spp. have been tested in assays for feeding deterrence and are usually deterrent to generalist herbivores. Thus, the chemical defenses of cyanobacteria may play a critical role in bloom formation and persistence by limiting the grazing activity of potential consumers.

In addition to direct poisoning, cyanobacterial blooms cause aquatic toxicity indirectly by asphyxia from the organic mass produced (e.g., the seasonal die-off of a macroscopic bloom as photoperiod and light intensity diminishes in waterbodies in temperate latitudes) and from anoxia during decomposition of the organic matter. Aquatic cyanobacteria are unusual when compared with other aquatic biota in their ability to utilize ammonia as a source of nitrogen. They also utilize nitrate and nitrite for this if it is available, but some freshwater taxa will revert to forming heterocysts for energy-intensive nitrogen fixation if dissolved forms of nitrogen become growth-limiting. Because of their less efficient anaerobic metabolism, cyanobacteria cannot generally grow as quickly as their aerobic eukaryotic algal competitors in fresh waterbodies, but they may prevail either seasonally or continuously in eutrophic waterbodies lacking a strong current, and this appears to be a typical outcome if poor water quality conditions such as low dissolved oxygen, higher pH (e.g., 8.5 and higher), elevated ammonia concentrations, higher turbidity, and phosphate-laden sediments are also present.