Task 1. Literature Review and Database Development
Purpose: The purpose of this task is to develop a literature database on the human and aquatic health impacts of *Microcystis* that will be available to the public via the Office of Environmental Health Hazards Assessment website.

Method: Information will be obtained from the current literature through library and internet sources to assess the potential acute and chronic impact of *Microcystis* toxicity on humans and wildlife. Medline, Aquatic Sciences and Fisheries abstracts and other relevant databases will be searched for articles on conditions that stimulate algal blooms, the forms of microcystin toxins as well as their chemical and physical characteristics, the mechanisms of microcystin toxicity on fish, wildlife and humans; useful techniques for evaluating potential effects; and potential management options. This review will be used to refine the conceptual model of the impact of *Microcystis* on human and wildlife health (Figs. 3, 4, 5 and 6). This task will also produce a literature database for public access that will be posted on www.OEHHA.CA.GOV/ECOTOX that includes similar literature databases. These databases undergo an internal peer review process.

Task 2. *Microcystis* Biomass, Toxicity and Food Web Transfer
Task 2 a. *Microcystis* Biomass, Toxicity and Environmental Conditions Field Study and Historical Data Analysis.
Purpose: The purpose of this study is to determine the spatial and temporal variability of *Microcystis* biomass, toxicity and associated changes in physical, chemical and biological factors. Analyses will be used to refine the conceptual model of environmental factors that control *Microcystis* blooms in the Delta (Fig. 3) and the impact of Microcystis on ecosystem structure and function and human and ecosystem health (Figs. 4, 5 and 6) through an adaptively-managed and hypothesis-driven field program (Table 1).

Field sampling: Field research will be conducted to determine the spatial and temporal variability of the *Microcystis* bloom biomass and toxicity in the Delta and associated environmental conditions in relation to hypotheses needed to refine the current conceptual model on causal factors and humans and ecosystem impacts (Figs. 3 through 6; Table 1). During the first year water samples for biomass and toxicity will be collected semi-monthly sampling at 9 stations throughout the Delta between June and November. Stations will be selected to represent a broad spectrum of habitats characterized by differences in streamflow, residence time, water transparency, depth, salinity and vegetation type including shallow water habitat, deep river channel, flooded island, beach, emergent vegetation and submerged vegetation. The exact stations and sampling interval will depend on the data analysis of the 2004 research program that will be completed in summer 2005. Vertical profiles with a YSI 6600 sonde will provide information on water quality conditions such as water temperature, pH, specific conductance, dissolved oxygen and turbidity at the station as well as

the vertical gradient in chlorophyll *a* fluorescence, an estimate of phytoplankton biomass. Water samples will be collected to measure the chemical conditions at the station and will include nutrients (nitrate, ammonia, orthophosphate, total phosphorus and silica), organic constituents (total and dissolved organic carbon and trihalomethane (THM) formation), anions and cat ions (bromide, chloride, sulfate, magnesium and calcium) and biological community composition (phytoplankton species composition) and biomass (chlorophyll *a* concentration). The presence of dissolved microcystins in the water column will be also be assessed from 1-liter water samples. The growth potential and efficiency of the *Microcystis* among habitats will be assessed through *in situ* dissolved oxygen light and dark bottle primary productivity incubations at different light levels (Vollenweider 1974). Mesozooplankton for zooplankton community analysis will be obtained by a diagonal tow of a Clarke-Bumpus net (154 µm mesh) with an attached Oceanics current meter. Zooplankton samples will be immediately preserved with 5% buffered formalin with Rose-Bengal dye added. This sampling regime will be adaptively managed over the sampling period to address hypotheses that focus on habitats, time periods, environmental conditions and mechanisms that promote *Microcystis* growth and toxicity.

Laboratory analyses: Laboratory water quality analyses will be conducted using Standard Methods (APHA et al. 1998; U.S. EPA 1983) and conducted by the CA Department of Water Resources Chemical Laboratory at Bryte, an EPA certified laboratory. The sampling and laboratory procedures will follow procedures described in a QAMP and QAPP that will be developed for the project.

Data analysis: Data will be analyzed to assess spatial and temporal variability using graphical and tabular display and nonparametric and parametric statistical techniques as appropriate. Nonparametraic analyses will include single comparisons with the Wilcoxon Mann-Whitney-U and Spearman correlation and multiple comparisons with Kruskal-Wallis. Parametric analysis will include principal component analysis.

Analyses will be designed to address questions including: What is the current level of *Microcystis* bloom biomass and toxicity? What habitats contain the highest biomass and toxicity? How does biomass and toxicity vary with stream flow and salinity? How does the bloom biomass and toxicity vary seasonally? What environmental conditions are associated with high bloom biomass and toxicity? Is the bloom biomass or toxicity increasing over time? How does bloom biomass and toxicity vary among years or water year types? Does the local phytoplankton and zooplankton community composition vary with bloom biomass or toxicity? (Table 1).

Historical data analysis
Historical Estuarine Monitoring Program, State Water Project (MWD) and Interagency Ecological Program data from 1970 to present will be analyzed to

determine links between the occurrence of the *Microcystis* bloom since 1999 and environmental conditions including water quality conditions, residence time, and flow patterns throughout the impacted Delta region and reservoirs upstream. Historical data will also be analyzed to determine if there have been any changes in the phytoplankton and zooplankton community biomass and species composition since 1999 that could be linked to the appearance of the *Microcystis* bloom and associated water quality conditions. Comparison of *Microcystis* field data and historical monitoring data analyses will be made to refine the conceptual model of factors that control *Microcystis* blooms in the Delta (Fig. 3) and their impact on human and wildlife health and ecosystem function (Fig. 4 and 5).

Data analysis: Patterns in these data will be identified using parametric statistical techniques including cluster analysis, principal component analysis and analysis of variance. Information from these analyses will be displayed graphically and in tabular form.

Analyses will address questions including: Was there a change in the water quality, environmental conditions, or plankton community associated with the appearance of the bloom in the late 1990s? Are there any trends in the water quality, environmental or plankton data since 1999? ? Have changes in the Delta phytoplankton coincided with changes in reservoirs upstream? Can any of the changes in conditions since 1999 be used to better understand the field sampling data results or the reverse? Are there any changes or trends in environmental conditions associated with the bloom that can be used to assist management? What is the best way to monitor the bloom? (Table 1).

Food web toxicity and trophic transfer

The movement of microcystins into the tissues of animals in the food web will be measured at least two times during the season. Animals will initially be collected from four stations in the Delta and will include plankton (micro-, meso- and macro-zooplankton), epibenthic (amphipods), benthic (clams) and open water invertebrates (fish). Methods for collection will include a vertical pump collection for microzooplankton, a diagonal net (154 µm mesh) tow for mesozooplankton, a diagonal net (505 µm) tow for macrozooplankton, hand picking from vegetation and ponar dredge for epibenthos, ponar dredge for benthos and species specific nets or left-over tissue for fish. Whole animals in the case of plankton, epibenthos and benthos and muscle and liver tissue in the case of fish will be immediately frozen until analysis for microcystins. Inland silversides and threadfin shad will be the initial focus of the fish tissue study because of their abundance, wide distribution throughout impacted areas of the Delta, plankton diet and frequent use by predators. The tissue of species that have declined in recent years such as Delta smelt and striped bass will also be tested using fish muscle and liver

tissue obtained from preexisting sampling programs so there will be no impact on take permits.

Adaptive management will again be used to adjust field sampling each year to address hypothesis about toxicity (Table 1) needed to refine conceptual models on toxic impacts on ecosystem structure and function (Fig. 6) including questions that address trophic transfer mechanisms (e.g., animals with high microcystins concentration and predators with highly contaminated prey). Toxicity analyses will be conducted as part of Task 2b described below.

Data analysis: Data will be analyzed to determine spatial and temporal differences using graphical and tabular display and nonparametric and parametric statistical techniques as appropriate. Nonparametraic analyses will include single comparisons with the Wilcoxon Mann-Whitney-U and Spearman correlation and multiple comparisons with Kruskal-Wallis. Parametric analysis will include principal component analysis.

Analyses will be designed to address basic questions about Microcystis food web toxicity including: What is the current level of *Microcystis* bloom toxins in each trophic level of the food web? How does the microcystins content of the food web vary with season and water year? What are the pathways of movement of microcystins into the food web and how does this vary seasonally and among water years? What habitats contain the highest food web toxicity? What environmental conditions are associated with high food web toxicity? Does the local phytoplankton and zooplankton community composition vary with microcystins concentration in the food web? (Table 1).

Task 2 b. *Microcystis* Biomass, Food web and Environmental Water Toxicity
Purpose: The purpose of this task is to determine the toxicity of Microcystis biomass and the concentration of Microcystis toxins in the food web organisms. Results from these analyses will be combined with information gained from Task 2a and 3 to refine conceptual models of the impact of *Microcystis* toxicity on humans and ecosystem structure and function (Figs. 4 and 6).

Algal tissue method - Filters for toxin analysis in *Microcystis* biomass will be extracted by sonication with 10 ml of 50% methanol containing 1 % acetic acid, clarified by centrifugation, and the extract used for analysis of the different toxins. Total particulate microcystins concentration in algal tissue will be initially assessed using the protein phosphatase inhibition assay (PPIA) technique. Assays will be run in 96-well plates containing 0.1 mU enzyme (recombinant protein phosphatase 1A, catalytic subunit, Roche Applied Science), 1.05 mg para-nitrophenyl phosphate (Sigma Biochemical) and 10 µl of sample or microcystin-LR (Sigma Biochemical) using the method of Carmichael and An (1999). The rate of phosphate hydrolysis will be calculated from the change in

absorbance at 405 nm over 1 h and compared to the control (no added microcystin-LR) and standards containing between 6 and 40 µg l^{-1} microcystin-LR. Blanks (no enzyme, no toxin), unknowns, standards, and controls will be run in duplicate. The PPIA assay is an activity based assay whose results correlate well with the biological activity of the total microcystins. Duplicate analysis will also be done using a structure based ELISA assay as part of the routine quality control. For dissolved microcystins, a replicate 1-L water sample is lyophilized to dryness without prior filtration. The dried powder is then extracted with methanol and assayed as described above, and the dissolved microcystin fraction determined by difference.

Samples with the highest levels of total microcystins (> 0.5 µg L^{-1}) will be further analyzed by high pressure liquid chromatography to identify the specific microcystins in the sample. Samples will be separated using a Dupont Ace 4.6 x 250 5µ C18 column and a two-step linear gradient of 30 to 70% acidified acetonitrile to acidified water at 0.8 ml/min (Harada, 1995). Detection will be either mass selective using electrospray ionization (LCMS, Agilent 1100 series MSD) or by UV absorbance using a Water's model 996 photodiode array detector (PDA) between 210 and 300nm. For LCMS, all ions between 900 and 1250 amu will be combined to form the total ion chromatograph and potential microcystins identified on the basis of their molecular ions and retention times. For PDA detection, microcystins will be identified on the basis of having an absorbance maximum at 239 nm in their UV spectrum and their retention times.

Animal tissue method - Zooplankton tissue will be lyophilized and then extracted by sonication with 10 ml of 50% methanol containing 1 % acetic acid, clarified by centrifugation, and the extract used for analysis of toxins. Clam and fish tissue will be extracted using a 50% acidified methanol in a Waring Blender. To determine the efficiency of extraction, representative samples of each tissue type are split and purified microcystin-LR, microcystin-YR, microcystin-RR are added to lyophilized or homogenized tissue. Prior experiments showed that the recovery of toxins from algal samples was greater than 95% of microcystins. In contrast, the recovery of toxins from clam tissue was 50-60% of the added toxins. Tissue samples are will be analyzed using both the PPIA and ELISA assay to eliminate potential false positives. In addition, LCMS will be used to determine the individual microcystin distribution and to find and quantify known microcystin degradation products such as the glutathione derivative. These methods were effective for sampling done in 2003 (Lehman et al. in press).

Task 3. Fish Bioassay
Purpose: The purpose of this experiment is to evaluate the potential chronic impacts of microcystin on growth performance and health status of splittail by a long term feeding study and evaluate the effect by histopathology. The information will be used to refine the conceptual model of *Microcystis* toxicity on humans and the ecosystem (Figs. 4 and 6).

Methods: Diet preparation - Five test diets containing graded levels of microcystins found in the Delta food web zooplankton will be used. The study will focus on microcystin-LR, one of the most toxic microcystins in the Delta bloom. Demethyl microcystin-LR , the most abundant microcystin in the Delta bloom, will be evaluated once it is characterized or through concentrated algal samples. Splittail responses to diets containing 1 to 5 µg dry *M. aeruginosa* /g dry weight will be determined using the chronic toxicity method described by Teh et al. (2004a). This species is useful because it can be successfully grown in long-term bioassay studies (Teh et al. 2004b) and is a good analogue for other native species. The control diet will be formulated with no supplementation and contain vitamin free casein 310 g kg^{-1}, wheat gluten 150 g kg^{-1}, egg albumin 40 g kg^{-1}, dextrin 299 g kg^{-1}, non-nutritive bulk 35g kg^{-1}, carboxymethyl cellulose 20 g kg^{-1}, cod liver oil:corn oil (1:1) 80 g kg^{-1}, vitamin mix 10 g kg^{-1}, mineral mix 30 g kg^{-1}, choline chloride 4 g kg^{-1}, santoquin 0.19 g kg^{-1}.

Fish and experimental conditions - Splittail juveniles (5-7 g) will be reared from the natural spawning of captive broodstocks at the Center for Aquatic Biology and Aquaculture, University of California, Davis. The experiment will be conducted in an indoor flow-through system with 24 individual tanks (40 fish per tank, 66cm in diameter, 90L water in volume). Prior to the initiation of the experiment, 40 fish will be randomly distributed into each tank and acclimated with basic diet (without microcystin) for 2 weeks. Water temperature will be maintained at 18-20 °C during the experimental period. The flow rate of individual tanks will be 4 L min^{-1}. The system will be kept under a natural photoperiod. Test diet will be randomly assigned to three replicate tanks. Fish will be fed twice daily (9:00am and 4:00pm) with a daily feeding rate of 3 % body weight (BW). Feces and uneaten feeds in the tanks will be cleaned by siphon 30 minutes after feeding and mortality will be recorded daily. The feeding study will last for 1 year or when 50% mortality rate is observed. All moribund and dead fish will be weighed, measured, and processed for histopathological analysis.

Sample collection - Six fish from each tank will be randomly collected at the end of 10, 20, 40, and 60 weeks of exposure. Each fish will be individually netted, examined for gross deformities, and euthanized with an overdose of 3-aminobenzoic acid ethyl ester (MS-222, Sigma, St. Louis, Missouri, USA). Fish will be measured (total length) and body and liver weighed to determine conditional and liver somatic indices. Livers will be examined grossly for neoplasms and then sub sampled for histopathology evaluation. A sub sample of muscle, liver, and gastro-intestinal tract will also be frozen in liquid nitrogen and stored at -80°C for microcystin analysis by Dr. Boyer (Task 2b).

Health assessment - The condition factor and organo-somatic indices have been used extensively as indicators of the well-being of fish health and population assessments (Schmitt and Dethloff 2000). Gross measurements and weights will

be used to determine condition (CI) and hepatosomatic (HSI) indices in fish. CI is a measure of "plumpness" and defined as (body weight in grams)(100) / length in cm^3. HSI is the liver to body weight ratio. Both indices are broad measures of general health. Changes in CI reflect alterations in growth and nutritional status and changes in HSI reflect sex, sexual maturity, or general health and nutrition. Contaminant-induced alterations of somatic growth will therefore be reflected by these indices. Hepatosomatic (100 X liver weight/body weight) indices have proven to be sensitive and simple indicators of responses when compared in fishes from contaminated versus reference sites. For example, hepatosomatic (HSI) and gonadosomatic indices (GSI) decreased in fishes living in water receiving pulp and paper mill effluent (Jobling et al., 1996).

Histopathology has been recommended as a physiological approach to pollution investigation (Sindermann 1985) and has been used with both fish and aquatic invertebrates for the assessment of contaminant-mediated adverse effects (Adams et al. 1999). Histologic lesions can be examined in a tissue specific manner, allowing identification of target organs for specific xenobiotic compounds (Wester and Canton 1986). Direct histological damage of the liver and gonads has been documented in several studies involving heavy metals, pesticides, and pulp and paper mills effluents (Singh et al. 1994; Teh et al. 1997, 1999, 2004a, 2000b, 2005). Liver will be subjected to histopathology as described in Teh et al. (2004a; 2000b; 2005). Lesions will be qualitatively scored based on severity (e.g., 0= normal or no lesion; 10= mild or less than 10% of the organ is affected, 20= moderate or 10-50% of the liver is affected, and 30= severe or > 50% of the liver is affected).

Feasibility - The proposed research experiment will be done at two locations on the campus of UC Davis: the Center of Aquatic Biology and Aquaculture (CABA) and the Aquatic Toxicology Program laboratory (ATP). CABA is equipped with a 1500 sq. ft. Quonset hut and 800 sq. ft. laboratory. The Quonset hut also has a flow-through freshwater system, supplying 19 ± 2 °C well water at a rate of up to 400 L/min to 72 small (90 L) and 6 large (700 L) fiberglass tanks. The CABA lab and animals are daily by personnel and continuously by remote sensing.

Data analysis: Data will be analyzed using SAS software. All data will be subjected to one-way analysis of variance (ANOVA) to determine treatment effects on growth, condition factor, HSI, and chemical concentrations in tissues. If significant differences ($p<0.05$) were identified, differences among means will be compared by the Duncan's multiple comparison. Analyses will be used to refine conceptual models of the impact of *Microcystis* toxicity on human and ecosystem structure and function as described in Figs. 4 and 6.

Products from the project will include a BDAT data base containing all new field and laboratory data with a meta database for Tasks 2 and 3 and a literature database on the OEHHA website for Task 1. Programmatic semi-annual status

reports and a final report will summarize the findings of the project to CALFED. The final report will include a discussion of the current and future impact of *Microcystis* on the Delta and restoration projects and any needed direction for future work. At least two peer reviewed journal articles will be prepared as a result of this project.

This hypothesis-driven field and experimental research project will substantially enhance our understanding of the cyanobacterial bloom of *Microcystis* in the Sacramento-San Joaquin Delta thereby helping us to prioritize our future monitoring and research efforts in regard to the impacts of microcystin biomass and contaminants in the Bay-Delta Ecosystem on human health and ecosystem function. The study will also yield critical information on the long-term impact of microcystin toxicity on at-risk fish species and human health needed to develop long-term restoration management strategies.

Research Program Feasibility: This research program will be fully implementable once contracts are in place. The tasks will not be limited by weather, delay from other projects, environmental compliance or permitting.

Management Plan: Dr. P. Lehman will oversee contract management through the CA Department of Water Resources, be the primary contact, synthesize semi-annual status report and final summary report information, track program progress, and conduct the field sampling program. Project integration will proceed through internet discussions and collaboration among scientists through toxicity analyses conducted by Dr. Boyer on field and laboratory samples prepared by both Dr. Teh and Dr. Lehman. Information from the literature database will be used by all research investigators and information from all tasks will be synthesized to refine conceptual models by Dr. Lehman.

Data Management: All data from the field and laboratory studies will be entered into the Interagency Ecological Program BDAT database and CERES meta database in order to allow access to the public, government and stakeholder groups. Citations from the literature review will be listed on the OEHHA website and be linked to the Interagency Ecological Program.

Information Dissemination: Information from this study will be disseminated to the scientific research community through oral and poster presentations at local scientific meetings including the CALFED Science Conference, Interagency Ecological Program Annual Meeting and State of the Estuary Conference and national meetings such as the American Society of Limnology and Oceanography, Estuarine Research Federation and the American Fishery Society. Information will be available to the public in the CALFED final report and the international scientific community through three journal articles.

Schedule: The entire project will run from June 2006 to July 2008. Task 1: June 2006 to Dec 2006. Task 2: June 2006 to July 2008. Task 3: June 2006 to July 2008.

Budget: The cost for the three year project is $500,000. In addition, the State University of New York will add an additional $7,483 in cost share in salary and benefits over the three year period ($3668 year 1; $3815 year 2).

Permitting: Fish are not captured with the *Microcystis* or zooplankton nets. Any fish used will be obtained through IEP monitoring programs.

3. Project Justification

CALFED justification: This hypothesis-driven field and experimental research on this toxic cyanobacterial bloom in the Delta is related to the CALFED Programmatic Record of Decision (ROD) 1) Water Quality Program goals to improve water quality in the Delta by reducing the load of organic carbon, reducing the load of unknown toxins and reducing the impairment from oxygen-demanding substances; 2) Ecosystem Restoration Program goals to improve and increase aquatic habitat, improve ecological function in the Delta and contribute to the recovery of key species and 3) Water storage and use efficiency goals to manage water in wildlife areas and provide reliable good quality water through storage programs as included in the Delta Improvements Package Plan.

The proposal directly addresses the Science PSP goals to learn about 1) Processes Controlling Delta Water Quality such as improving water quality monitoring, the origin of organic carbon sources and causes of oxygen-demanding substances and 2) Relative Stresses on Key Fish Species, especially plankton feeders such as Delta smelt, including impacts on food quantity and quality, predation processes, contaminant effects and habitat and 3) Assessment and monitoring including phytoplankton and shallow water habitat.

Funding is needed from CALFED to continue research on the *Microcystis* bloom in the Delta because no future Interagency Ecological Program Estuarine Monitoring Program funding is available due to the State and Federal budget shortfalls. *Microcystis* is not sampled by the routine Estuarine Monitoring Program because it requires special sampling techniques and sampling at depths and stations outside of the mandated program.

Research justification: *Microcystis biomass - Microcystis* is a cyanobacteria (bluegreen algae) that can form extensive surface scums (Fig. 2). In some cases, this organism can also form a harmful algal bloom (HAB) through production of potent hepatotoxins. A bloom of a large colonial form of *Microcystis* first appeared throughout the Delta in 1999 and has occurred every year since between June and November (Lehman and Waller 2003; Lehman et al. in press) and may be an invasive species. A single day of sampling in October

2003, indicated the bloom was distributed throughout the Delta as far north as Collinsville on the Sacramento River, as far south as navigation light 13 and 14 on the San Joaquin River and the State Water Project (SWP) and Central Valley Project (CVP) water export facilities on Old River and as far seaward as Martinez (Fig. 1; Lehman et al. in press). This region has been routinely monitored by the CA Department of Water Resources (DWR) and U. S. Bureau of Reclamation (USBR) Estuarine Monitoring Program (EMP) since the 1970s, but *Microcystis* was not found in the Delta during this time (IEP database) and the *Microcystis* genus was not recorded for Delta phytoplankton communities in the Delta between 1975 to 1982 (Lehman and Smith 1991).

Microcystis is an extremely cosmopolitan and common freshwater cyanobacterium that blooms in eutrophic lakes and reservoirs throughout the world including the United States, Canada, Australia, New Zealand, South Africa and Japan (Carmichael 1995; Downing et al. 2001: Paerl 1988; Watanabe 1995). It has also been observed in estuaries including the Neuse River estuary (Paerl 1988) and the Potomac River estuary (Sellner et al. 1993) in the USA, the Swan River estuary in Western Australia (Robson & Hamilton 2003) and the Patos Lagoon Estuary in Southern Brazil (Yunes et al. 1996).

The organic carbon loading from the bloom to the Delta may be high. In October 2003, near the end of the bloom, chlorophyll *a* concentration for the largest *Microcystis* colonies (> 75 µm diameter) alone reached 0.075 μgL^{-1} and reached 554 $\mu g\ L^{-1}$ in concentrated 1-minute net tow samples. The chlorophyll *a* concentration of the non-Microcystis phytoplankton community in the region ranged from 1 to 3 $\mu g\ L^{-1}$. In 2004, bi-weekly sampling conducted between July and November at nine stations indicated the chlorophyll *a* biomass of large (> 75 µm diameter) *Microcystis* colonies was 4 times higher in September 2004 than those measured in October 2003 and were highest in shallow freshwater habitat.

Microcystis Toxicity - Blooms of *Microcystis* often form surface scums that impede contact recreation sports, reduce aesthetics, lower dissolved oxygen concentration and cause taste and odor problems in drinking water (Carmichael 1995). Some *Microcystis* blooms also contain hepatotoxic microcystins that cause liver tumors and cancer in wildlife and humans (Carmichael 1995).

The *Microcystis* bloom in the Delta formed a surface scum that contained toxic microcystins (Fig. 2). One surface water sample collected in the Delta in 2000 contained total microcystins concentration of 0.8 and 1 µg nmicrocystin-LR equivalents L^{-1}, near or at the World Health Organization (WHO) advisory level of 1 $\mu g\ L^{-1}$ (World Health Organization 1998; Lehman and Waller 2003). All of the algal biomass samples collected on the single sampling day in October 2003 contained microcystins, including the highly toxic microcystin-LR. Ambient total microcystins concentration in the largest colonies reached 0.016 $\mu g\ L^{-1}$ and was well below the WHO advisory level, but concentrations in 1-minute concentrated

net tow samples reached 119 µg L^{-1}, orders of magnitude above the advisory level. Small quantities of microcystins were also found in animal tissue; 1 to 3.5 µg microcystins (g dry weight)$^{-1}$ in zooplankton tissue and 0.02 µg microcystins (g dry weight) $^{-1}$ in clam tissue (Lehman et al. in press). *Microcystis* is not readily eaten by zooplankton or clams (Sellner et al. 1993), but toxins can bioaccumulate. Tissue of the cladocera *Bosmina* spp. contained microcystins concentration that was 202% higher than in the co-occurring algal tissue (Park & Watanabe 1995).

The impact of *Microcystis* toxicity on humans and wildlife in the Delta is unknown. *Microcystis* in the Delta contained a number of microcystins, each with their own toxicity. The toxicity of the most abundant microcystin in the Delta bloom, tentatively identified as a demethyl microcystin-LR, is unknown and cannot be ascertained until the molecule is purified and individually characterized. Full characterization of this molecule is in progress (G. Boyer personal communication). Microcystin-LR comprised 9 to 23% of the total microcystins and is a powerful hepatotoxin associated with both acute and chronic liver damage (Kaya 1995). This microcystin is associated with toxicity to birds and fish and is suspected as a cause of human cancer in China and Australia (Carmichael 1995; Kaya 1995). Other microcystins in the Delta bloom include microcystin-WR (7-13%), microcystin-FR (5-8%) and microcystin-RR (0-4%).

The toxicity data available so far for July through August 2004 indicated microcystins concentration in concentrated 1-minute net tows ranged from 0 to >500 µg microcystin-LR equivalents L^{-1}, indicating that another toxic bloom occurred in the Delta in 2004. No information is available on the potential impact to human health and ecosystem structure and function in the Delta.

The potential adverse impact of *Microcystis* on the estuary is large. Water from the northern region is used for drinking water and irrigation and the region is an important recreational area for sport fishing and water contact sports. The estuary is habitat for many anadromous commercial and recreational fish including striped bass and Chinook salmon and is a feeding ground for marine mammals. The estuary also contains many threatened or endangered aquatic organisms including the fish Delta smelt, winter run Chinook salmon and Sacramento splittail. Many of these endangered fish species are declining (California Bay-Delta Authority 2000). Of great concern are the low Delta smelt and striped bass population indices measured over the last four years (www.delta.dfg.ca.gov). These low indices could not be explained by the usual causal factors such as hydrology and *Microcystis* may be a contributing factor.

Fish toxicity - Numerous experimental studies have documented the toxicity of microcystin exposure through gastrointestinal or blood circular systems on fish such as common carp (Fischer and Dietrich 2000; Li et al. 2001), gold fish (Xu et al. 1998), Atlantic salmon (Williams et al. 1997), rainbow trout (Bury et al. 1997;

Fischer et al. 2000), channel catfish (Zimba et al. 2001), brown trout (Bury et al. 1997), Northern snakehead (Chen et al.1995), and grass carp (Chen et al. 1995; Zhang et al. 1996). The cyprinids appeared to be more susceptible to microcystin toxic effects than salmonids (Tencala 1995). The above studies were limited to acute toxic experiments, and using an approach based on either oral gavaging, intraperitoneal or dorsal aorta injection, which cannot reflect the uptake route under natural environments. Little is known about the chronic impacts of microcystins except for a study of the effect of microcystins on silver carp through a sub-chronic toxicity experiment (Xie et al. 2004).

The long term health effects of native at-risk fish chronically exposed to microcystins in the Delta is unknown. No study has investigated the impact of *Microcystis* on splittail, which declined in population over its habitat in the estuary. Sacramento splittail, a cyprinid endemic benthivore, is now largely confined to the San Francisco Estuary and was listed as threatened species by the U.S. Fish and Wildlife Service. Splittail are opportunistic daytime benthic foragers that feed on opossum shrimp, earthworm, clams, insect larvae, and other invertebrates. Detrital material also makes up a high percentage of their stomach contents (Meng and Moyle 1995). A recent study revealed that splittail are changing their diet preference to consume a large quantity of clams and benthic invertebrates as their major food source (Feyrer et al. 2003). Microcystin exposure via trophic transfer warrants investigation for this species directly and as an analogue for other species in the estuary such as Delta smelt and striped bass that have declined in the Delta for unknown reasons in the past four years.