CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

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MEMORANDUM

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FROM: Lauren Zeise, Ph.D.
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Lauren Zeise (Jun 15, 2022 15:27 PDT)

DATE: June 15, 2022

SUBJECT: RECOMMENDATIONS FOR ACUTE NOTIFICATION LEVELS FOR ANATOXIN-A, CYLINDROSPERMOPSIN, MICROCYSTINS AND SAXITOXINS

In response to a request by the State Water Resources Control Board ("Water Board"), the Office of Environmental Health Hazard Assessment (OEHHA) is recommending health-based acute (one day) notification levels (NLs) for four cyanotoxins in drinking water: anatoxin-a, microcystins, cylindrospermopsin and saxitoxins. The recommended levels are shown in Table 1. Information on the sources and nature of these toxins is provided in the previous memoranda (OEHHA, 2021a,b).

These acute (one day) NLs are the recommended maximum water concentrations that humans can consume over a 24-hour period but not longer. These recommendations are supplementary to the short-term (up to one month or three months) NL recommendations provided to the Water Board in two memoranda on May 3, 2021. These short-term values are also shown in Table 1.

An acute (one day) NL recommendation for saxitoxins was also provided to the Water Board in the May 3, 2021 memorandum; a short-term recommendation was not developed for this toxin. Based on additional information discussed in this memorandum, OEHHA is updating the acute saxitoxins NL recommendation.

Table 1. Recommended acute and short-term NLs for cyanotoxins[a]

Chemical	Acute NL[b] (duration)	Health effect (peer-reviewed study)	Short-term NL[a,b] (duration)	Health effect (peer-reviewed study)
Anatoxin-a	8[c] (up to 1 day)	Neurotoxicity (Fawell et al., 1994; 1999a)	4 (up to 1 month)	Neurotoxicity (Fawell et al., 1999a)
Cylindro-spermopsin	3 (up to 1 day)	Liver & kidney cell death (Bazin et al., 2010)	0.3 (up to 3 months)	Liver damage (Chernoff et al., 2018)
Microcystins	3[c] (up to 1 day)	Liver damage (Chernoff et al., 2021)	0.03 (up to 3 months)	Decline in sperm number (Chen et al., 2011)
Saxitoxins[a]	0.5[c] (up to 1 day)	Neurotoxicity (EFSA, 2009)	NA	NA

[a] Recommendations for short-term NLs and the one-day NL of 0.6 µg/L for saxitoxins were provided to the Water Board May 3, 2021: https://oehha.ca.gov/water/crnr/notice-availability-notification-level-recommendations-four-cyanotoxins-drinking-water.
[b] In units of micrograms per liter (µg/L), which is equivalent to parts per billion (ppb).
[c] NL is for the total concentration of the toxin variants.

ANATOXIN-A

The derivation of the anatoxin-a acute NL recommendation involved a search of the toxicological and epidemiological literature. PubMed was searched using the terms anatoxin with acute, single, oral, gavage, and drink in various combinations, which resulted in 58 articles. Out of those references, three acute oral toxicity studies were found. Each of these studies was considered of sufficient quality for further evaluation as the basis for a recommended NL. PubMed was also searched for epidemiological or human case studies on anatoxin-a using the terms anatoxin with human, poison, food, drink, ingest, consum* in various combinations, which resulted in 35 articles. One of these references involved human poisonings where anatoxin-a was measured at significant concentrations in the seafood consumed (Bire et al., 2020). However, there was not enough information to use this study in the development of an acute NL.

The applicable studies were evaluated to determine the most appropriate study of sufficient quality for establishing a health-protective concentration, and identification of a dose that could be consumed by sensitive people without adverse effects. This dose was then converted to a drinking water concentration, considering drinking water consumption rates by sensitive populations. The resulting health-protective

concentration is recommended as the acute NL for anatoxin-a. These steps are explained in further detail below.

Selection of Critical Study and Point of Departure

Few acute, oral exposure studies exist for anatoxin-a. Fawell and colleagues performed a 5-day oral gavage study in Crl:CD-1 (ICR)BR (VAF plus) mice (2 males and 2 females per dose) that was described in a study report (Fawell and James, 1994) and a journal publication (Fawell et al., 1999a). This study showed a good dose-response relationship, where 1.2 and 2.5 milligrams chemical per kilogram body weight per day (mg/kg-day) of anatoxin-a had no observed effects, 6.2 mg/kg-day was the minimum lethal dose (1 of 4 mice died), with hyperactivity in surviving mice, and 12.3 mg/kg-day resulted in total mortality (4 of 4 mice died). The Fawell et al. (1994; 1999a) 5-day oral study has a lowest-observed-adverse-effect level (LOAEL) of 6.2 mg/kg-day and a no-observed-adverse-effect level (NOAEL) of 2.5 mg/kg-day based on mortality. This study has limitations, including the low number of animals tested and the lack of a control dose. However, a 10-day developmental toxicity screening study was described in the same report, which used the same strain of mice and the same chemical. This study exposed 10-12 pregnant mice/dose to 0 or 2.5 mg/kg-day anatoxin-a by gavage for 10 days, on gestation days 6-15. The authors recorded bodyweights, clinical signs, and numbers of live and dead implantations in maternal mice. Fetuses were weighed, sexed and examined for external abnormalities. No effects were observed in this study and 2.5 mg/kg-day was again the NOAEL.

Two oral exposure, median lethal dose (LD_{50}) studies are available, each using single administrations of varying doses of anatoxin-a. Puddick et al. (2021) reported an oral LD_{50} of 10.6 mg/kg via gavage and 25 mg/kg via feeding in female Swiss albino mice. Stevens and Krieger (1991) reported an oral LD_{50} of 13.3 mg/kg in male Swiss Webster mice. The unknown dose levels of anatoxin-a and incomplete reporting of observed effects leave too much uncertainty to base an acute NL on these LD_{50} studies. However, these studies lend some support to using the NOAEL of 2.5 mg/kg-day.

Based on the dose-response data from the 5-day study by Fawell et al. (1994; 1999a), OEHHA selected the NOAEL of 2.5 mg/kg-day as the acute point of departure (POD) for anatoxin-a.

Acceptable Daily Dose Determination

OEHHA is deriving an acute acceptable daily dose (ADD_{Acute}), based on the 5-day oral mouse study by Fawell et al. (1994; 1999a), as the estimated maximum dose (in mg/kg-day) of anatoxin-a that can be consumed by humans for one day without toxic effects. Although the critical study is a 5-day study, OEHHA recommends applying the ADD_{Acute} to a one-day exposure, rather than 5 or more days, because all the animals that died did so within minutes of exposure.

To determine the ADD_{Acute}, the POD is divided by factors that account for variability and uncertainties in the risk assessment, such as differences between animals and humans, and variability among humans in response to anatoxin-a.

An ADD_{Acute} is calculated as follows:

$$ADD_{Acute} = POD \div UF_{Combined}, \qquad \text{(Equation 1)}$$

where,

ADD_{Acute}: The acute acceptable daily dose is the estimated maximum dose of a chemical that can be consumed by humans for one day without toxic effects. The ADD_{Acute} is expressed as mg/kg-day.

POD: The point of departure is the dose of a chemical from a study in animals or humans that is used as a starting point for calculation of the ADD. The POD is expressed as mg/kg-day.

$UF_{Combined}$: The combined uncertainty factor (unitless) is the composite of factors used to address the variability and uncertainty in deriving the ADD_{Acute}. The separate UFs (e.g., for interspecies extrapolation and for intraspecies variability) are multiplied to determine the combined UF for acute exposure.

To determine the ADD_{Acute}, the NOAEL of 2.5 mg/kg-day from the 5-day oral study in mice by Fawell et al. (1994; 1999a) is selected as the POD. A UF of 10 is used for interspecies extrapolation, accounting for possible differences in the way laboratory animals and humans respond to a chemical. A UF of 30 is used for intraspecies variability, which accounts for differences in the way humans, including sensitive populations, respond to a chemical. A UF of $\sqrt{10}$ is used to account for the limited toxicity database. The $UF_{Combined}$ for acute exposure to anatoxin-a is the product of these three UF values, or 1,000.

Thus, an ADD_{Acute} can be calculated for anatoxin-a as shown below:

$$ADD_{Acute} = 2.5 \text{ mg/kg-day} \div 1{,}000 = 0.0025 \text{ mg/kg-day}.$$

Acute Health-Protective Concentration Determination

Following the determination of the ADD_{Acute}, the acute health-protective concentration (C_{Acute}) of anatoxin-a in drinking water can be derived by incorporating the drinking water intake (DWI) rate and the relative amount of the ADD_{Acute} that is ingested from tap water (the relative source contribution or RSC).

$$C_{Acute} = ADD_{Acute} \times RSC \div DWI \qquad \text{(Equation 2)}$$

where,

ADD_{Acute}: The acceptable daily dose or estimated maximum dose of a chemical that can be consumed by humans for one day without toxic effects. The ADD_{Acute} is expressed as mg/kg-day.

RSC: The relative source contribution is the proportion of the ADD_{Acute} that can come from tap water, as part of total exposure from all sources (including food and air). The RSC value is determined based on available environmental monitoring data.

DWI: The daily water intake rate expressed as liters per kilogram of body weight per day (L/kg-day).

Infants may be particularly sensitive to neurotoxicity and they have a higher drinking water intake rate adjusted for body weight than adults. To calculate a health-protective concentration for infants, the drinking water intake rate of 0.237 L/kg-day is used as it is the average rate for infants 0 to 6 months of age (OEHHA, 2012). The RSC is set to one because a formula-fed infant is not expected to be exposed to anatoxin-a from any sources other than the tap water used to reconstitute formula. Thus, an acute health-protective concentration for infants can be calculated as shown below:

$$C_{Acute} = (0.0025 \text{ mg/kg-day} \times 1) \div 0.237 \text{ L/kg-day}$$

$$= 0.01055 \text{ mg/L, or } 11 \text{ µg/L (rounded).}$$

In addition to drinking water, children and adults can be exposed to anatoxin-a from consumed fish and shellfish and from recreational water activities. Anatoxin-a appears to have a high bioconcentration factor (BCF) in fish like rainbow trout (Osswald et al., 2011), indicating that fish can effectively bioconcentrate anatoxin-a from water. Furthermore, the *California Tribes Fish-Use: Final Report* (Shilling et al., 2014) noted that the 95th percentile rates of consumption of caught fish ranged between 30 g/day and 240 g/day. A consumption rate of 142 g/day, equivalent to four to five fish meals per week, is considered to represent a high level of consumption. Based on this, OEHHA estimated that children or adults consuming very high amounts (100-200 g within 24 hours) of fish and shellfish potentially contaminated with anatoxin-a would obtain most of their exposure from these sources and not from drinking water, thus supporting the use of an RSC of 0.2 for drinking water. Among these groups, children (2-16 years old) would be considered more susceptible than adults due to a slightly higher drinking water intake (0.061 L/kg-day). An acute health-protective concentration for children 2-16 years old can be calculated as shown below:

$$C_{Acute} = (0.0025 \text{ mg/kg-day} \times 0.2) \div 0.061 \text{ L/kg-day}$$

$$= 0.0082 \text{ mg/L, or } 8 \text{ µg/L (rounded).}$$

The acute health-protective concentration calculated for children is lower than that calculated for infants and is chosen as the acute NL recommendation for anatoxin-a.

When data are available for anatoxin-a variants (homoanatoxin-a, dihydroanatoxin-a and dihydrohomoanatoxin-a) OEHHA recommends applying this acute NL to the total concentration measured for anatoxin-a and the variants. The variant homoanatoxin-a has the same mode of action as anatoxin-a, a postsynaptic depolarizing neuromuscular blockade, and shows similar toxicological potency. For example, both chemicals have similar LD_{50} values by intraperitoneal injection (Skulberg et al., 1992; Wonnacott et al., 1992). The variant dihydroanatoxin-a has recently been shown to be more toxic than anatoxin-a in one LD_{50} study (Puddick et al. 2021). However, due to the limitations discussed above, more testing would be needed for toxicity criteria to be established (Puddick et al. 2021).

The New Zealand Ministry of Health used an intraperitoneal LD_{50} for homoanatoxin-a with an uncertainty factor of 5,000 to develop an acute Provisional Maximum Acceptable Value of 2 µg/L in drinking water (New Zealand, 2019). It is unclear whether this is a one-day guideline. It appears that no other government agency has developed a drinking water guideline for anatoxin-a based on acute toxicological data. OEHHA did

not develop a guideline for homoanatoxin-a because there are no oral toxicity data for this chemical. As mentioned above, OEHHA recommends applying the acute NL of 8 µg/L to all anatoxin-a congeners based on the NOAEL of 2.5 mg/kg-day from the 5-day oral exposure of anatoxin-a to mice (Fawell et al. 1994; 1999a).

MICROCYSTINS

The derivation of the acute NL recommendation for microcystins involved a search of the toxicological and epidemiological literature. PubMed was searched using the terms microcystin with acute, single, oral, gavage, ingest in various combinations, which resulted in 43 articles, with four acute oral toxicity studies using purified toxin. PubMed was also searched for human microcystin poisonings using the terms microcystin with humans, epidemiol*, poison, food, ingest*, consump*, “drinking water” in various combinations, which resulted in 226 articles. No acute human poisoning cases were suitable for use in the development of the acute microcystin NL.

The four acute oral toxicity studies were appraised to determine the most appropriate study of sufficient quality for establishing a health-protective concentration, and identification of a dose that could be consumed by sensitive people without adverse effects. The chosen dose was then converted to a drinking water concentration, considering drinking water consumption rates by sensitive populations. The resulting health-protective concentration is recommended as the acute NL for microcystins. These steps are explained in further detail below.

Selection of Critical Study and Point of Departure

Chernoff et al. (2021) exposed 12 – 48 male and female BALB/c mice per dose to MC-LA (microcystin-LA). The single gavage doses of MC-LA administered were 0, 0.5, 1, 3, and 7 mg/kg, and animals were observed for 24 hours. The authors recorded several biochemical and gross effects. Measures of hepatotoxicity included liver score (gross visual anomalies of congestion/hemorrhage, infiltration of glycogen or lipid, reticulated pattern of the lobules), serum enzyme markers of liver damage (alanine amino transferase (ALT), aspartate amino transferase (AST), and glutamate dehydrogenase (GLDH)), liver/body weight ratios, and serum bilirubin levels. Significant moribundity incidence (hunching, non-responsiveness to interaction, lethargy, hypothermia, diarrhea, and/or weight loss greater than 10%) was also recorded.

This study provided a good dose-response relationship, with the highest dose of 7 mg/kg resulting in 63% of the mice being moribund with multiple biochemical and gross

effects ($p \leq 0.001$). The next dose, 3 mg/kg, resulted in increased liver scores, liver enzymes (ALT, AST, and GLDH), liver/body weight ratios, and serum bilirubin levels, indicating liver toxicity ($p \leq 0.001$ to $p \leq 0.05$). The two lowest doses, 1 and 0.5 mg/kg, showed no observable adverse effects. The LOAEL for the Chernoff et al. (2021) study is 3 mg/kg and the NOAEL is 1 mg/kg. This study also tested MC-LR, MC-LY, MC-RR, and MC-YR, and these congeners had higher LOAELs.

In an older study by Fawell et al. (1999b), five male and five female mice (Cr1:CD-1[ICR]BR strain [VAF plus]) were given a single gavage dose of MC-LR at 0.5, 1.58 and 5 mg/kg. No control dose was given in this study. Animals were observed for a period of 14 days following the single dose of MC-LR. The authors recorded body weight, microscopic observations of liver, kidneys, lung, spleen and adrenal glands, mortality, and clinical signs in decedents.

The Fawell et al. (1999b) study showed a good dose-response with the highest dose of 5 mg/kg resulting in mortality (5 of 10 mice) and hemorrhage/necrosis in the liver (10 of 10 mice). The dose of 1.58 mg/kg gave the minimum lethal dose (1 of 10 mice died) and resulted in histopathological liver damage (diffuse or centrilobular hemorrhage in 4 of 10 mice), while the lowest dose of 0.5 mg/kg showed no observable adverse effects. OEHHA identified a LOAEL of 1.58 mg/kg for microscopic liver changes and mortality, and a NOAEL of 0.5 mg/kg.

Both Chernoff et al. (2021) and Fawell et al. (1999b) are good studies that support each other in terms of showing liver toxicity at similar levels. Although Fawell et al. (1999b) reported the lowest LOAEL (1.58 mg/kg), Chernoff et al. (2021) is a more robust study that tested more animals, used more dose levels, and recorded a variety of endpoints. Thus, OEHHA chose Chernoff et al. (2021) as the critical study.

OEHHA used the US EPA's Benchmark Dose Software (BMDS version 3.2[1]) to model the dose-response relationship of the data in Chernoff et al. (2021). BMDS uses mathematical models to determine the dose (benchmark dose or BMD) that corresponds to a pre-determined level of response (benchmark response or BMR). To account for uncertainty in the data, the model also calculates the 95% lower confidence limit of the BMD, known as the BMDL (L stands for lower confidence limit). OEHHA modeled several endpoints, with the most sensitive being increased relative liver weight in female mice exposed to MC-LA. Because a 10% increase in relative liver weight is generally accepted as biologically significant, OEHHA modeled the Chernoff et al.

[1] Available at: https://www.epa.gov/bmds/benchmark-dose-software-bmds-version-3

(2021) data for increased relative liver weight using a BMR of 10% relative deviation. The model with the best fit[2] for the MC-LA data produced a $BMDL_{10}$ of 1.01 mg/kg, which was selected as the acute POD.

Other studies in the literature support the use of the acute POD of 1.01 mg/kg. Mrdjen et al. (2018) orally exposed mice to MC-LR for 7 days and had a freestanding LOAEL of 3 mg/kg-day for liver histopathology and increased liver enzymes. Fawell et al. (1999b) orally exposed pregnant mice during gestation days 6 – 15 and had a LOAEL of 2 mg/kg-day and a NOAEL of 0.6 mg/kg-day for mortality, macroscopic changes in maternal liver, and embryotoxicity. Yoshida et al (1997) showed an oral LD_{50} of 10.9 mg/kg; while Fawell et al. (1999b) found an approximate oral LD_{50} of 5 mg/kg. Overall, there are a limited number of oral exposure acute toxicity studies for microcystins.

Acceptable Daily Dose Determination

To determine the ADD_{Acute} for microcystins, the $BMDL_{10}$ of 1.01 mg/kg-day from the single dose oral study in mice by Chernoff et al. (2021) is selected as the POD. The $UF_{Combined}$ for acute exposure to microcystins is the product of the UF of 10 for interspecies extrapolation, accounting for possible differences in the way laboratory animals and humans respond to a chemical, the UF of 30 for intraspecies variability, which accounts for differences in the way humans, including sensitive subpopulations, respond to a chemical, and the UF of $\sqrt{10}$ for the limited toxicity database. Using Equation 1, the ADD_{Acute} can be calculated for microcystins as shown below:

$$ADD_{Acute} = 1.01 \text{ mg/kg-day} \div 1{,}000 = 0.00101 \text{ mg/kg-day}.$$

Acute Health-Protective Concentration Determination

Following the determination of the ADD_{Acute}, the acute health-protective concentration (C_{Acute}) for microcystins in drinking water can be derived by incorporating the DWI and RSC. Applying the 0 to 6 month infant drinking water intake rate of 0.237 L/kg-day and RSC of 1 in Equation 2, C_{Acute} for infant exposure can be calculated as:

$$C_{Acute} = (0.00101 \text{ mg/kg-day} \times 1) \div 0.237 \text{ L/kg-day}$$

$$= 0.0043 \text{ mg/L, or } 4 \text{ µg/L (rounded)}.$$

[2] The model with the best fit (visual fit, lowest Akaike information criterion (AIC), and significant p-values for goodness of fit) was Polynomial Degree 3.

Unlike infants, children and adults can be exposed to microcystins from consuming fish and shellfish obtained from contaminated water and from recreational water activities. Although microcystins demonstrate a relatively low bioaccumulation potential in fish, concentrations of microcystins in shellfish, particularly mussels, can be very high as a result of harmful algal blooms (Testai et al., 2016). OEHHA estimated that exposure to microcystins from consuming fish and shellfish for children or adults can occasionally be much greater than the exposure from drinking water at the recommended NL level, necessitating the use of an RSC of 0.2. Among these groups, children (2-16 years old) would be considered more susceptible than adults due to a slightly higher drinking water intake (0.061 L/kg-day). An acute health-protective concentration for children 2-16 years old can be calculated as shown below:

$$C_{Acute} = (0.00101 \text{ mg/kg-day} \times 0.2) \div 0.061 \text{ L/kg-day}$$

$$= 0.0033 \text{ mg/L, or } 3 \text{ µg/L (rounded).}$$

The acute health-protective concentration calculated for children is lower than that calculated for infants and is chosen as the acute NL recommendation for microcystins. OEHHA recommends the C_{Acute} of 3 µg/L as the acute NL for microcystins in drinking water. This recommended acute NL is expected to be protective of sensitive subpopulations from all routes of exposure for acute exposures up to one day.

OEHHA also recommends applying the acute NL to the total of all variants of microcystins that are measured. MC-LA is common in California and is one of the most toxic microcystin variants tested. The toxicity of some of the other microcystin variants is lower (Chernoff et al., 2021). However, based on toxicological limits of the scientific literature and the limits in monitoring microcystins in surface waters, the acute NL based on MC-LA should be applied to the sum of all microcystin variants.

No other government agency appears to have developed a drinking water guideline for microcystins based on acute toxicological data.

CYLINDROSPERMOPSIN

To develop the acute NL for cylindrospermopsin, a search of the toxicological and epidemiological literature was conducted. PubMed was searched using the terms: cylindrospermopsin AND acute. This search resulted in 38 articles. PubMed was also searched for epidemiological or human case studies on cylindrospermopsin using the terms: cylindrospermopsin AND human AND poison. This search resulted in 101 articles. In addition, one article was identified from a book chapter that was outside of the PubMed database searches.

From this search, OEHHA identified two candidate critical studies for the development of an acute NL recommendation for cylindrospermopsin (Bazin et al., 2010; Shaw et al., 2001), which will be discussed below. The rest of studies were excluded due to reasons such as missing critical information (e.g., quantitative information on dose or response), publications were not acute toxicity studies (e.g., they were reviews or biomonitoring or exposure studies), lack of dose-response, lack of controls, etc.

Selection of Critical Study and Point of Departure

Bazin et al. (2010) treated male Swiss mice (3/dose group) with a single oral gavage administration of 0, 1, 2, or 4 mg/kg cylindrospermopsin (98% purity). Tissue samples were removed for histological examination and micronucleus and alkaline comet assays 24 hours after treatment. DNA damage was observed in bone marrow and colon as demonstrated by the alkaline comet assay (ACA) while cell death was observed in kidney, liver, duodenum and jejunum by histological examination. Kidney and liver were the target organs of cylindrospermopsin and a NOAEL of 1 mg/kg was identified by OEHHA for the histological changes in the liver and kidney.

Shaw et al. (2001) studied effects of cylindrospermopsin on white Quackenbush mice. Cylindrospermopsin was extracted and purified from cyanobacteria *Cylindrospermopsis raciborskii*. In one experiment from this study, mice (4/dose group) were given a single oral gavage dose of cylindrospermopsin at 0, 1, 2, 4, 6, or 8 mg/kg. Liver was a primary target of cylindrospermopsin toxicity, with fatty vacuolation and hepatocyte necrosis observed in the liver of cylindrospermopsin-treated mice. The authors developed a TDI (tolerable daily intake) of 0.04 µg/kg/day from the single oral exposure study. Their TDI was calculated by using a combined UF of 25,000 (10 for interspecies extrapolation, 10 for intraspecies variability, 10 for less than lifetime dose, 5 for single dose, 5 for use of a LOAEL). The authors used the following equation to calculate the TDI:

$$TDI = (NOAEL \text{ or } LOAEL) \div UF$$

Although the authors considered 1 mg/kg a LOAEL in calculating the TDI, they did not present any information about the critical effect on which it was based. The data table in the study showed that no animal reached morbidity until the second highest dose (6 mg/kg), though changes in pathology of the liver were described at lower doses. Furthermore, the number of animals with histopathological changes in the liver was not reported for any dose. The lack of information from the Shaw et al. (2001) study made it difficult to identify the POD and therefore made the study less reliable, though it still provides support for the liver effects observed in Bazin et al. (2010), which was chosen as the critical study.

Acceptable Daily Dose Determination

To determine the ADD_{Acute} for cylindrospermopsin, OEHHA selected the NOAEL of 1 mg/kg from Bazin et al. (2010) as the POD and applied a $UF_{Combined}$ of 1,000. It includes a UF of 10 for extrapolating from rodents to humans, 30 to account for variability between humans, and $\sqrt{10}$ for database deficiencies. Using Equation 1, the ADD_{Acute} can be calculated for cylindrospermopsin as shown below:

$$ADD_{Acute} = 1 \text{ mg/kg-day} \div 1{,}000 = 0.001 \text{ mg/kg-day}.$$

Acute Health-Protective Concentration Determination

Following the determination of the ADD_{Acute}, the acute health-protective concentration (C_{Acute}) of cylindrospermopsin in drinking water can be derived by incorporating the DWI and RSC. As with anatoxin-a and microcystins, the 0 to 6 month infant drinking water intake rate of 0.237 L/kg-day and RSC of 1 are applied for cylindrospermopsin. Using Equation 2, C_{Acute} for cylindrospermopsin for infant exposure can be calculated as:

$$C_{Acute} = (0.001 \text{ mg/kg-day} \times 1) \div 0.237 \text{ L/kg-day}$$

$$= 0.0042 \text{ mg/L, or } 4 \text{ µg/L (rounded)}.$$

In addition to drinking water, children and adults can be exposed to cylindrospermopsin from consumed fish and shellfish and from recreational water activities. Similar to anatoxin-a, cylindrospermopsin appears to have a high BCF, indicating that fish are able to bioconcentrate cylindrospermopsin from water (Scarlett et al., 2020). OEHHA estimated that children or adults consuming very high amounts (100-200 g within 24

hours) of fish and shellfish potentially contaminated with cylindrospermopsin would obtain most of their exposure from these sources and not from drinking water, thus supporting the use of an RSC of 0.2 for drinking water. Among these groups, children (2-16 years old) would be considered more susceptible than adults due to a slightly higher drinking water intake (0.061 L/kg-day). An acute health-protective concentration for children 2-16 years old can be calculated as shown below:

$$C_{Acute} = (0.001 \text{ mg/kg-day} \times 0.2) \div 0.061 \text{ L/kg-day}$$

$$= 0.0033 \text{ mg/L, or } 3 \text{ µg/L (rounded).}$$

The acute health-protective concentration calculated for children is lower than that for infants and is chosen as the acute NL recommendation for cylindrospermopsin.

Because the POD, adopted from Bazin et al. (2010), is based on a single dose rodent study, OEHHA recommends a duration of one day for the health-protective concentration of 3 µg/L for cylindrospermopsin.

The New Zealand Ministry of Health used an intraperitoneal LD_{50} for cylindrospermopsin with an uncertainty factor of 5,000 to develop an acute Provisional Maximum Acceptable Value of 1 µg/L in drinking water (New Zealand, 2019). It appears that no other government agency has developed a drinking water guideline for cylindrospermopsin based on acute toxicological data.

SAXITOXINS

In the NL recommendations provided to the Water Board on May 3, 2021, OEHHA calculated an ADD of 0.15 µg STXeq/kg for saxitoxins based on the acute POD of 1.5 µg STXeq/kg (LOAEL for neurotoxic effects) from EFSA (2009). The health-protective concentration was then calculated based on the infant exposure scenario from reconstituted formula, using an RSC of 1 and 0 to 6 month infant daily drinking water intake of 0.237 L/kg-day:

$$C_{Acute} = (0.00015 \text{ mg/kg-day} \times 1) \div 0.237 \text{ L/kg-day}$$

$$= 0.00063 \text{ mg/L, or } 0.6 \text{ µg/L (rounded).}$$

However, similar to other cyanotoxins, very high levels of saxitoxins can be occasionally detected in food, particularly in shellfish (EFSA, 2009; Oyaneder Terrazas et al., 2017). While infants do not consume fish or shellfish, children and adults do, and the possibility

of co-exposure to saxitoxins in drinking water and occasional high levels in food cannot be excluded, thus supporting the use of an RSC of 0.2 for drinking water. Among these groups, children (2-16 years old) would be considered more susceptible than adults due to a slightly higher drinking water intake (0.061 L/kg-day). An acute health-protective concentration for children 2-16 years old can be calculated as shown below:

$$C_{Acute} = (0.00015 \text{ mg/kg-day} \times 0.2) \div 0.061 \text{ L/kg-day}$$

$$= 0.00049 \text{ mg/L, or } 0.5 \text{ µg/L (rounded).}$$

The acute health-protective concentration calculated for children is lower than that for infants and is chosen as the acute NL recommendation for saxitoxins. This value supersedes the previous acute NL recommendation of 0.6 µg/L.

Because the POD adopted from EFSA (2009) is based on case reports of human poisoning through consumption of contaminated shellfish, OEHHA recommends a duration of one day for the health-protective concentration of 0.5 µg/L for saxitoxins. WHO (2020) developed an acute drinking water guidance value for saxitoxins of 3 µg SXTeq/L based on the same EFSA (2009) POD. This value is higher than the proposed acute NL recommendation due to different assumptions in risk assessment.

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