CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

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MEMORANDUM

TO: Darrin Polhemus
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FROM: Lauren Zeise, Ph.D. Lauren Zeise
Lauren Zeise (May 3, 2021 16:13 PDT)
Director

DATE: May 3, 2021

SUBJECT: ANATOXIN-A NOTIFICATION LEVEL RECOMMENDATION

Recommendation

In response to a request by the State Water Resources Control Board, the Office of Environmental Health Hazard Assessment (OEHHA) is recommending a health-based short-term notification level of 4 micrograms per liter (µg/L), equivalent to 4 parts per billion (ppb), for anatoxin-a in drinking water.[1] This means the chemical at (but not above) the recommended level can be consumed by humans for up to one month without toxic effects.

Background on Anatoxin-a

Anatoxin-a is a cyanotoxin produced by cyanobacteria, which live in most aquatic ecosystems. Approximately 41 species of pelagic or benthic cyanobacteria have been shown to produce anatoxin-a (Christensen and Khan, 2020; Testai et al., 2016). Under certain environmental conditions, cyanobacteria increase rapidly in number and create a harmful bloom, which may make the water toxic to human health and to animals through the production of cyanotoxins.

Anatoxin-a has been found in waters of California and has caused the death of many domesticated animals in the state (Backer et al., 2013; Conklin et al., 2020; Kelly et al.,

[1] Derived from the following peer reviewed study: Fawell JK, Mitchell RE, Hill RE, Everett DJ (1999). The toxicity of cyanobacterial toxins in the mouse: II anatoxin-a. Hum Exp Toxicol 18(3): 168-173. https://journals.sagepub.com/doi/10.1177/096032719901800306?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed&

2019; Puschner et al., 2010). It can also contaminate drinking water supplies (US EPA, 2020).

Toxicities Observed After Accidental Exposure to Anatoxin-a

Anatoxin-a is a neurotoxin in mammals and is rapidly absorbed from the gastrointestinal tract (Carmichael et al., 1975; Stevens and Krieger, 1991). It acts as a potent nicotinic cholinergic agonist causing muscle twitching, reduced movement, labored breathing, loss of coordination, gasping, convulsion, and even death (Carmichael et al., 1975; Carmichael et al., 1979; Aronstam and Witkop, 1981; Swanson et al., 1986; Fitzgeorge and Clark, 1994). Symptoms occur rapidly and animals that survive generally return to normal status (Fitzgeorge and Clark, 1994; Stevens and Krieger, 1991; Carmichael et al., 1979).

In humans, there have been three confirmed cases of food poisoning with anatoxin-a after eating Sea Figs (*Microcosmus* sp.) (Biré et al. 2020; Schmitt et al., 2019). Patients reported difficulty with focusing, double vision, impaired coordination, dizziness, tinnitus, and cramping in the legs and abdomen. These symptoms resolved within 3 to 24 hours. The poisonings were confirmed based on high levels of anatoxin-a in the leftovers of the meals and the absence of other classical marine toxins. Also, there were fifteen additional food poisoning cases following consumption of Sea Figs, between 2011 and 2018, where the patients had similar symptoms but leftovers were not measured for toxins (Schmitt et al., 2019).

Derivation of Health-Protective Concentration

The derivation of the anatoxin-a NL recommendation began with a comprehensive search of the toxicological and epidemiological literature. The literature was then appraised to determine the most appropriate study for establishing a health-protective concentration. The subsequent steps took into account variability and uncertainty to identify a dose that could be consumed by sensitive people without adverse effects. This dose was then converted to a drinking water concentration, taking into account drinking water consumption rates by sensitive populations. The resulting health-protective concentration is recommended as the NL for anatoxin-a. These steps are explained in further detail below.

Selection of Critical Study and Point of Departure

OEHHA searched the scientific literature for anatoxin-a toxicity in early 2020 and found two repeated-exposure animal toxicity studies that could be used for dose-response evaluation: a short-term study in rats exposed through drinking water and a short-term study in mice exposed orally by gavage. No human studies were identified that could

serve this purpose because human exposures are published as case reports consisting of single exposures to anatoxin-a in contaminated seafood, with no dose-response data.

The study in rats exposed groups of 20 female Sprague Dawley rats to anatoxin-a in drinking water for seven weeks (Astrachan and Archer, 1981; Astrachan et al., 1980). Approximate exposure rates of anatoxin-a (partially-purified) were 0, 0.05 and 0.5 milligrams per kilogram of body weight per day (mg/kg-day). Endpoints evaluated throughout the study included clinical signs, food consumption, body weight, red and total white blood cell counts and blood chemistry (4 serum enzyme activities). At the end of the exposure, endpoints evaluated included histology (7 tissues), organ weights (3 organs) and gross pathology. No treatment-related effects were observed, indicating a free-standing no-observed-adverse-effect level (NOAEL) of 0.5 mg/kg-day. This study was not well-described and gave limited information on the study design and the observation of results.

In the short-term mouse study, Fawell et al. (1999) exposed 10 male and 10 female Crl:CD-1(ICR)BR (VAF plus) mice per dose by gavage daily for 4 weeks with anatoxin-a (purity not reported). Doses used were 0, 0.1, 0.5 and 2.5 mg/kg-day (rounded). There were no toxicologically significant differences between control and treated animals in histopathology (40 tissues), hematology (10 parameters) and blood chemistry (16 parameters). Dietary intake, body weight, organ weights and ophthalmoscopy results were comparable to those of controls (Fawell and James, 1994).

However, there were two unexplained deaths in the four-week gavage study (Fawell and James, 1994; Fawell et al., 1999). One male mouse died after receiving 0.5 mg/kg on day 10. Then, one female mouse died after receiving 2.5 mg/kg on day 14. Both of these animals died within 2.5 hours of the dose administration and were clinically unremarkable prior to death. Necropsy, hematology and blood chemistry findings were unremarkable, and there were no findings to indicate that an intubation error had occurred. These deaths were not dose-dependent and no adverse clinical signs were observed in the mice that survived. When a lethal dose of anatoxin-a is given, there are significant signs of neurotoxicity that precede death, such as gasps, tremors, muscular twitching, loss of coordination, immobilization and convulsions. These effects were not observed in the animals that died in this study. Additionally, animals receiving a lethal dose of anatoxin-a typically die within minutes of exposure.

In deriving its short-term health-based reference value ($HBRV_{short\text{-}term}$) for anatoxin-a, the World Health Organization (WHO) noted that necropsy did not show the cause of death in these animals, thus anatoxin-a toxicity could not be ruled out (WHO, 2020). However, OEHHA concluded that it was appropriate to assume these two deaths were not treatment-related because of the delayed onset of death, the lack of classic neurotoxic signs prior to death, the lack of a dose-response relationship, no signs of

toxicological impacts in the surviving animals, and supporting evidence from other oral exposure studies using 0.5 or 2.5 mg/kg-day that saw no effects (Fawell and James, 1994; Fawell et al., 1999; OEHHA, 2012a; Astrachan and Archer, 1981; Astrachan et al., 1980). Thus, OEHHA identified a free-standing NOAEL of 2.5 mg/kg-day for the Fawell et al. (1999) study.

OEHHA chose Fawell et al. (1999) as the critical study over the Astrachan et al. (1980) study because it was more detailed in its reporting and in the number of endpoints it examined, it tested both sexes, and gavage administration is more likely to induce neurotoxic effects for a fast-acting neurotoxin like anatoxin-a. In addition, the study tested a wider range of doses, and its exposure duration of 4 weeks is well suited to represent the short-term nature of anatoxin-a exposure through drinking water. Considering the dose-rate effect, one can argue the Fawell et al. (1999) study is more sensitive than the Astrachan et al. (1980) study.

Based on the dose-response data reported by Fawell et al. (1999), OEHHA selected the NOAEL of 2.5 mg/kg-day as the point of departure (POD) for anatoxin-a. This is consistent with OEHHA's approach for developing peer-reviewed anatoxin-a reference doses for health-based water concentrations for recreational exposures (OEHHA, 2012a). Considering that the minimum oral lethal dose (LD_{min}) is 6.1 mg/kg-day and the oral median lethal dose (LD_{50}) is 13.3 mg/kg-day in rodents, the POD of 2.5 mg/kg-day is only 2.4-fold lower than the minimum oral lethal dose. WHO made a conservative assumption that the animal deaths in the Fawell et al. (1999) study were treatment-related and selected 0.1 mg/kg-day as the NOAEL for derivation of its $HBRV_{short\text{-}term}$, but also noted that the NOAEL could be as high as 2.5 mg/kg-day (WHO, 2020).

Acceptable Daily Dose Determination

OEHHA is deriving a short-term acceptable daily dose (ADD_{ST}), based on the four-week oral mouse study, as the estimated maximum daily dose (in mg/kg-day) of the chemical that can be consumed by humans for up to one month without toxic effects. Anatoxin-a is an intermittent chemical in drinking water sources that is only present during and after harmful algal blooms. Furthermore, anatoxin-a has a half-life of approximately 14 days under normal light conditions at pH 8 or 10 (Smith and Sutton, 1993). Thus, an ADD_{ST} would be consistent with the episodic and short-term nature of the exposure scenario for anatoxin-a in drinking water.

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

An ADD_{ST} is calculated as follows:

$$ADD_{ST} = POD \div UF,$$

where the terms in the equation are defined as follows:

ADD_{ST}: The short-term acceptable daily dose is the estimated maximum daily dose of a chemical that can be consumed by humans for up to one month without toxic effects. The ADD_{ST} 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. A NOAEL of 2.5 mg/kg-day from a four-week oral study in mice (Fawell et al., 1999) is selected as the POD.

UF: The uncertainty factor (UF) is the culmination of factors used to address the variability and uncertainty in deriving the ADD_{ST}. For anatoxin-a, 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 subpopulations, respond to a chemical; and a UF of 10 is used to account for the very limited toxicity database and the fact that the POD is only 2.4-fold lower than the minimum oral lethal dose in rodents. The composite UF for anatoxin-a is 3,000 (unitless).

$$ADD_{ST} = 2.5 \text{ mg/kg-day} \div 3{,}000 = 0.00083 \text{ mg/kg-day}$$

The composite UF differs from the value used for the anatoxin-a reference doses previously developed by OEHHA (OEHHA, 2012a). OEHHA has since changed its default UF for intraspecies variability from 10 to 30, to adequately protect neonates and young infants from potential adverse effects of chemicals as evidence showed that human variation is often greater than 10-fold (OEHHA, 2012b).

Short-Term Health-Protective Concentration Determination

Following the determination of the ADD_{ST}, the short-term health-protective concentration (C_{ST}) of anatoxin-a in drinking water can be derived by incorporating the drinking water intake (DWI) of the chemical and the relative amount of the chemical derived from tap water (the relative source contribution or RSC).

$$C_{ST} = ADD_{ST} \times RSC \div DWI,$$

where,

C_{ST}: The short-term health-protective concentration in drinking water. The C_{ST} is expressed as milligrams of chemical in a liter of drinking water (mg/L).

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

RSC: The relative source contribution is the proportion of exposures to a chemical attributed to tap water, as part of total exposure from all sources (including food and air). The RSC values typically range from 20% to 80% (expressed as 0.20 to 0.80), and are 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).

Because infants may be particularly sensitive to neurotoxicity and they have a higher drinking water intake rate adjusted for body weight than adults, they are the most sensitive population for analysis. The drinking water intake rate of 0.237 L/kg-day is used in this assessment as it is the average rate for infants 0 to 6 months of age (OEHHA, 2012b). The relative source contribution (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, a short-term health-protective concentration can be calculated as shown below:

$$C_{ST} = (0.00083 \text{ mg/kg-day} \times 1) \div 0.237 \text{ L/kg-day}$$

$$= 0.00351 \text{ mg/L, or } 4 \text{ µg/L (rounded).}$$

If a short-term notification level were to be adopted for homoanatoxin-a, which is a homologue of anatoxin-a, OEHHA would also recommend 4 µg/L. 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; Lilleheil et al., 1997).

Conclusion

OEHHA recommends the short-term health-protective concentration of 4 µg/L as the NL for anatoxin-a in drinking water. In comparison, the WHO $HBRV_{short\text{-}term}$ for infants and children is 6 µg/L and for adults is 30 µg/L, because infants can consume up to 5 five-fold more drinking water than adults relative to body weight (WHO, 2020). The recommended NL is expected to be protective of sensitive subpopulations from all routes of exposure for short-term exposures up to one-month.

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