Provisional Health Advisory Level for Anatoxin-a and Saxitoxin in Drinking Water

Washington State Department of HEALTH

AUGUST 2024

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DOH 334-552 August 2024

Recommendation

Herein the Washington State Department of Health (DOH) evaluated the health risks associated with drinking water contaminated with anatoxin-a or saxitoxin. DOH has previously issued guidance for recreational exposure to anatoxin-a and saxitoxin. However, drinking water guidance is needed because of the increasing occurrence of harmful algal blooms in Washington State and presence of these toxins in freshwater systems that are often sources of drinking water.

Based on our evaluation, DOH recommends the following:

- A provisional health advisory level of 0.3 micrograms per liter (µg/L) for anatoxin-a in drinking water.
- A provisional health advisory level of 0.2 µg/L for saxitoxin in drinking water
- A Do Not Drink order be issued while drinking water sources exceed these concentrations.

Background

Cyanobacteria, commonly known as Blue-Green Algae, are found globally in both fresh and saltwater systems. Generally, cyanobacteria serve an important function in aquatic ecosystems fixing elements, such as nitrogen, into biologically active forms. However, some cyanobacteria can produce cyanotoxins which are harmful to both humans and wildlife. When environmental conditions are correct a rapid growth of algae can occur within waterbodies and this phenomenon is called a bloom. Harmful Algal Blooms (HABs) occur when the bloom is of toxin producing cyanobacteria and results in waterbodies having high concentrations of cyanotoxins for short time periods. Although there are several cyanotoxins that can be present in HABs, this assessment focuses on two: anatoxin-a and saxitoxin.

Anatoxin-a

Anatoxin-a is a naturally occurring bicyclic amine alkaloid produced by various freshwater cyanobacteria. Anatoxin-a was discovered in the early 1960s after a series of livestock deaths occurred in Canada (Gorham 1964). It was determined that these animals were watered from a shallow lake, which was experiencing a significant algal bloom at the time (Gorham 1964). This incident spawned research aimed at further understanding HABs and cyanotoxins; ultimately leading to the discovery of anatoxin-a (James et al. 2007). Since its discovery anatoxin-a has been identified as the cause of death in other mass wildlife and livestock death events and has been shown to be acutely lethal to domestic dogs (James et al. 2007). The primary health concern associated with anatoxin-a exposure is neurotoxicity. Anatoxin-a acts as a potent neuromuscular blocking agent, disrupting nerve impulse transmission (Carmichael 1989). In humans, anatoxin-a is known to cause gastrointestinal distress, muscle twitching, cramping and has been implicated in one death (Weirich and Miller 2002).

There are two primary routes of human exposure to anatoxin-a: 1) incidental ingestion during recreation activities and, 2) directly from consumption of contaminated drinking water. Recreational guidance for anatoxin-a and other cyanobacteria was previously published by DOH in 2008 (Hardy 2008). However, anatoxin-a occurrence is increasing throughout Washington State and the associated risk of impacts to surface water supplies used as drinking water necessitates further evaluation and guidance to protect public health.

Saxitoxin

Saxitoxin are a class of neurotoxins produced by marine dinoflagellates and some cyanobacteria. More than 30 saxitoxin analogues have been discovered, however, as saxitoxin is the most potent, the term saxitoxin is used to discuss all analogues within the group. Additionally, laboratory reporting is usually expressed as saxitoxin-equivalence to include all analogue concentrations. Saxitoxin is commonly associated with shellfish, specifically paralytic shellfish poisoning (PSP). Since its discovery, saxitoxin monitoring in shellfish has been important to ensuring food safety and much is known about detecting saxitoxin and saxitoxin effects in humans. Saxitoxin binds to sodium channels in axons and blocks nerve impulses (Henderson et. al. 1973). This gives rise to symptoms of paralysis such as tingling in the mouth and extremities and, if enough is ingested, paralysis of major muscle systems including those of the chest and abdomen, which may be life-threatening (Kao 1993).

Humans are typically exposed to saxitoxin through the consumption of shellfish. However, saxitoxin can be found in freshwater reservoirs and has been found historically in Washington State lakes albeit at low frequency (Trainer and Hardy 2015). This allows for exposure pathways identical to that of anatoxin-a (incidental ingestion during recreation and ingestion of contaminated drinking water). DOH developed recreational guidance for saxitoxin in 2011 (Hardy 2011), but the increasing frequency of HABs and detections in freshwater bodies necessitates establishing criteria for drinking water.

Review of Health Protective Values

Anatoxin-a

DOH performed a review of existing guidance and the primary toxicity studies for anatoxin-a. Research into the toxicity of anatoxin-a is limited with few oral toxicity studies having been performed. DOH identified two critical studies that covered acute exposure via the oral route, Astrachan and Archer (1981) and Fawell et al. (1999). Below includes summaries of both the critical studies and the evaluations performed by the EPA, the WHO, and the Oregon Health Authority.

Critical Studies

Astrachan and Archer (1981) was a 7-week drinking water study in rats. Dose groups ranged from 0 parts per million (ppm), 0.5 ppm, and 5 ppm anatoxin-a with no observed changes in body weight, food consumption, or serum enzymes observed in any dose group. From these results a no observed adverse effect level (NOAEL) of 0.5 mg/kg-day was determined. However,

this study had limitations including purity of the anatoxin-a, few observed potential toxic endpoints, and a freely accessible drinking water source instead of an oral gavage dose.

Fawell et al. (1999) was a 28-day drinking water study in male and female mice. Dose groups ranged from approximately 0, 0.1, 0.5 and 2.5 mg/kg-day anatoxin-a with no observed changes in body weight, food consumption, organ weight, or histopathology in any dose group. Minor changes in mean cell hemoglobin were observed in both treated males and females, however, it was determined that this was of no toxicological significance. One male in the 0.5 mg/kg-day dose group and one female in the 2.5 mg/kg-day dose group died during the study. The animals were unremarkable upon examination and necropsy and there was no way to determine cause of death, therefore, the authors concluded that death due to anatoxin-a could not be completely ruled out and a NOAEL of 0.1 mg/kg-day was determined.

EPA Evaluation

In 2015 the US EPA reviewed the available data to develop a health-based value for anatoxin-a. Included within the review were Astrachan and Archer (1981) and Fawell et al. (1999), however, as the studies deviated in both design and results and no subsequent studies have been performed to independently verify the findings, the EPA concluded that the data do not support the derivation of a reference dose (RfD).

WHO Evaluation

In 2020 the WHO developed “bounding values” for anatoxin-a for use in their Guidelines for Drinking Water Quality (WHO 2020). While not a formal guideline, the “bounding values” are intended to be used by risk assessors to inform public safety. The critical study for the WHO assessment was Fawell et al. (1999) selecting approximately 100 µg/kg-day as the NOAEL due to the ambiguity associated with animal deaths in the higher dose groups. The WHO however utilized two uncertainty factors of 10 each for interspecies and intraspecies variability. Assuming a 60 kg body weight and 2 L/day water consumption resulted in a bounding value of 30 µg/L for adults. A bottle-fed infant bounding value of 6 µg/L was derived by dividing the adult value by five to account for the higher water consumption to body weight ratio in infants.

Oregon Health Authority Evaluation

The Oregon Health Authority (OHA) selected the Fawell et al. (1999) study as the critical study. OHA also identified a NOAEL of approximately 100 µg/kg-day due to the ambiguity of deaths in the higher dose groups. However, OHA used three uncertainty factors of 10 for interspecies variation, intraspecies variation, and limited data availability, resulting in a tolerable daily intake of 0.1 µg/kg-day. Similar to the WHO, OHA calculated two health-based guideline values to account for the greater difference of water consumption to body size ratio between children and adults. For adults a body weight of 80 kg and 2.5 L/day water consumption resulted in a guideline value of 3 µg/L and for children using a body weight normalized ingestion rate of 0.15 L/kg-day resulted in a guideline value of 0.7 µg/L.

Saxitoxin

DOH performed a review of existing guidance and the primary toxicity studies for saxitoxins. DOH identified one critical study from the European Food Safety Authority (EFSA 2009) and three supporting studies: Arnich and Thebault 2018, Finch et. al. 2021, and Finch et. al. 2023. Below includes summaries of the studies and the evaluations performed by the WHO and the OHA. The EPA, to date, has not yet evaluated saxitoxins in terms of their risk to drinking water sources as they have done for microcystins, cylindrospermopsin, and anatoxin-a.

Critical Studies

The EFSA 2009 study had two components: a review of relevant animal studies for the development of toxic equivalency factors (TEF) for select saxitoxin analogues and an evaluation of human epidemiologic data of PSP cases with confirmed saxitoxin cause. Although the epidemiologic data focused on saxitoxin risk associated with the consumption of contaminated shellfish, the oral route of exposure from ingestion of shellfish is expected to be similar to that of drinking water. The epidemiologic evaluation considered human poisoning data from over 500 individuals and identified a lowest observed adverse effect level (LOAEL) of 1.5 µg/kg. As several individuals did not experience effects until much higher doses and as the data set included a wide variety of ages, an uncertainty factor of three was applied to derive a NOAEL of 0.5 µg/kg.

Arnich and Thebault 2018 is a human epidemiologic modeling study of PSP cases with confirmed saxitoxin cause. The evaluation reviewed 16 studies and included 143 individuals with the model predicting that a dose of 0.37 µg/kg would be sufficient to produce symptoms in 10% of people. However, of the studies included in the model, the true LOAEL identified was 1.8 µg/kg.

Finch et. al. 2021 and Finch et. al. 2023 are companion 21-day male and female mouse studies evaluating sub-acute exposure of saxitoxin via oral exposure. The 2021 study used a feeding-based dosing scheme whereas the 2023 follow up study used a bolus dose. While a feeding dose methodology better represents human exposure the authors noted that the exposure can still be variable even with modern methods for ensuring a homogenized food source and controlled feeding. Both studies identified no statistically significant differences in food consumption, growth, blood pressure, heart rate, motor coordination, grip strength, blood chemistry, hematology, organ weights or tissue histology for any dose group. The 2021 study had a high dose group of 730 µg/kg-day and the authors applied an uncertainty factor of 100 to account for interspecies differences producing a NOAEL of 7.3 µg/kg-day.

WHO Evaluation

In 2020 the WHO developed guidance values for saxitoxin for use in their Guidelines for Drinking Water Quality (WHO 2020). The critical study for the WHO assessment was EFSA 2009 selecting 1.5 µg/kg-day as the LOAEL and utilizing an uncertainty factor of 3 to account for usage of a LOAEL instead of a NOAEL to calculate a tolerable daily intake of 0.5 µg/kg-day. This in effect is identical to using the EFSA 2009 NOAEL of 0.5 µg/kg-day as both were derived

identically. WHO then calculated a guidance value for drinking water of 3 µg/L based on infant exposure assuming a 5 kg body weight and 0.75 L/day water.

OHA Evaluation

OHA selected the EFSA 2009 as the critical study and utilized a NOAEL for saxitoxin of 0.5 µg/kg-day. OHA also utilized an uncertainty factor of 10 due to the limitations in the database calculating a tolerable daily intake of 0.05 µg/kg-day. Identical to their methodology for anatoxin-a, OHA calculated two health-based guideline values to account for the greater difference of water consumption to body size ratio between children and adults. For adults a body weight of 80 kg and 2.5 L/day water consumption resulted in a guideline value of 1.6 µg/L. For children using a body weight-normalized ingestion rate of 0.15 L/kg-day, resulted in a guideline value of 0.3 µg/L.

WA DOH Recommendations

For anatoxin-a DOH selected Fawell et al. (1999) as the critical study for deriving a Tolerable Daily Intake (TDI). The gavage dosing scheme, inclusion of both males and females, as well as the broad set of histopathological and hematological analyses strengthens the confidence of this NOAEL. DOH agrees with the author findings that although there were no observed differences between dose groups it is impossible to rule out causality among the deaths observed in the 0.5 mg/kg-day and 2.5 mg/kg-day dose groups and therefore the selection of 0.1 mg/kg-day as the NOAEL is appropriate. For derivation of the TDI, DOH applied three uncertainty factors. Two factors of 10 for inter and intraspecies variability to account for potential differences in anatoxin-a sensitivity between humans and mice and variability among humans respectively. And an additional factor of 10 to acknowledge the limited oral toxicity data availability. Using the equation below we calculate a TDI of 0.1 µg/kg-day for anatoxin-a.

For saxitoxin DOH selected EFSA 2009 as the critical study for deriving a TDI using the NOAEL of 0.5 µg/kg-day in humans due to the epidemiologic data, quantity of data points, and demographic diversity. Additionally, the study by Arnich and Thebault showed a similar LOAEL (1.8 µg/kg) in their review. The epidemiologic data set is preferable to the animal extrapolated data of Finch et. al. 2021 and 2023, although the authors noted that the NOAEL they identified was within a factor of ten of the EFSA NOAEL and concluded that the current regulatory values appear appropriate. As the epidemiologic evidence for saxitoxin is associated with consumption of contaminated shellfish and illness due to PSP, infants are largely absent from this data set and therefore an uncertainty factor of 10 was used in the derivation of the TDI. Using the equation below we calculate a TDI of 0.05 µg/kg-day for saxitoxin.

$$TDI = \frac{NOAEL}{UF}$$

Where:

TDI = Tolerable Daily Intake (µg/kg-day)

NOAEL = No Observable Adverse Effect Level (µg/kg-day)

UF = Uncertainty Factors (unitless)

To derive a health advisory level (HAL), DOH selected formula fed infants as the most sensitive population due to their reliance on a single source of sustenance and higher water consumption per kg of body weight. DOH typically only provides guidance for the most sensitive population as all other populations would be protected at this level. The drinking water intake estimate of 0.290 L/kg-day used in the calculation is the 95th percentile of total direct and indirect water consumption for one- to three-month-old infants from Table 3.5 of the EPA Exposure Factors Handbook (EPA 2019). Using the equation below we calculated HALs of 0.3 µg/L for anatoxin-a and 0.2 µg/L for saxitoxin in drinking water.

$$HAL = \frac{TDI}{IR}$$

Where:

HAL = Health Advisory Level (µg/L)

TDI = Tolerable Daily Intake (µg/kg-day)

IR = Mass normalized ingestion rate (L/kg-day)

Conclusion

DOH recommends the following:

- A provisional health advisory level of 0.3 µg/L for anatoxin-a in drinking water.
- A provisional health advisory level of 0.2 µg/L for saxitoxin in drinking water
- A Do Not Drink order be issued while drinking water sources exceed these concentrations.

This level is set to be protective of sensitive subpopulations for a short-term exposure duration of approximately four weeks, which is considered adequate for a typical HAB event.

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