# Comprehensive Literature Review of Cyanotoxin Toxicity and Health-Based Screening Level Derivation

**AUGUST 19, 2021**

ToxStrategies

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# Comprehensive Literature Review of Cyanotoxin Toxicity and Health-Based Screening Level Derivation

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## Acronyms

| | |
|---|---|
| AGD | anogenital distance |
| ALP | alkaline phosphatase |
| ALT | alanine aminotransferase |
| AST | aspartate aminotransferase |
| ATX-a | anatoxin-a |
| BMD | benchmark dose |
| $BMD_{10}$ | benchmark dose—10% increase versus control |
| BMDL | benchmark dose level |
| $BMDL_{10}$ | benchmark dose level—lower one-sided 95% confidence interval for $BMDL_{10}$ |
| $BMDL_{1SD}$ | benchmark dose level—one standard deviation |
| BMDS | Benchmark Dose Software |
| CYN | cylindrospermopsin |
| DAF | dosimetric adjustment factor |
| dhATX-a | dihydroanatoxin-a |
| GD | Gestational Day |
| GGT | gamma glutamyl transaminase |
| HA | health advisory |
| HAB | harmful algal bloom |
| HED | human equivalent dose |
| HPG | hypothalamic-pituitary-gonadal |
| i.p. | intraperitoneal |
| $LD_{50}$ | lethal dose for 50 percent of the population |
| LDH | lactate dehydrogenase |
| LOAEL | lowest-observed-adverse-effect level |
| MC | microcystin |
| MC-LA | microcystin-LA |
| MC-LF | microcystin-LF |
| MC-LR | microcystin-LR |
| MC-LY | microcystin-LY |
| MC-RR | microcystin-RR |
| MC-YR | microcystin-YR |
| mg/kg | milligram per kilogram |
| mg/kg-day | milligram per kilogram per day |
| nAChRs | nicotinic acetylcholine receptors |
| N/A | not applicable/not available |
| NOAEL | no-observed-adverse-effect level |
| NOD | nodularin |
| NTP | National Toxicology Program |
| OECD | Organisation for Economic Co-operation and Development |

| | |
|---|---|
| OEHHA | California EPA's Office of Environmental Health Hazard Assessment |
| RfD | reference dose |
| RSC | relative source contribution |
| TCEQ | Texas Commission on Environmental Quality |
| TDI | tolerable daily intake |
| UCMR4 | Unregulated Contaminant Monitoring Rule 4 |
| UF | uncertainty factor |
| $UF_A$ | interspecies uncertainty factor from animal to human |
| $UF_D$ | database uncertainty factor |
| $UF_H$ | intraspecies variability factor |
| $UF_L$ | LOAEL-to-NOAEL uncertainty factor |
| µg/kg | microgram per kilogram |
| µg/kg-day | microgram per kilogram per day |
| µg/L | microgram per liter |
| USEPA | United States Environmental Protection Agency |
| WHO | World Health Organization |

## Executive Summary

ToxStrategies reviewed the existing health-based screening levels and toxicity factors for those cyanotoxins on the United States Environmental Protection Agency (USEPA) Unregulated Contaminant Monitoring Rule 4 (UCMR4) list, as well as for dihydroanatoxin-a (dhATX-a). The UCMR4 list of cyanotoxins included one cyanotoxin group (total microcystins [total MCs]) and nine cyanotoxins: anatoxin-a (ATX-a), cylindrospermopsin (CYN), microcystin-LA (MC-LA), microcystin-LF (MC-LF), microcystin-LR (MC-LR), microcystin-LY (MC-LY), microcystin-RR (MC-RR), microcystin-YR (MC-YR), and nodularin (NOD).

Existing human health-based screening levels, specifically drinking water screening levels for children and adults, from various US federal and state agencies as well international organizations, were identified and reviewed. Based on review of these existing health-based screening level values across regulatory entities, it was determined that they were generally developed using similar toxicity studies for each cyanotoxin. All MC drinking water screening levels are based on the congener MC-LR as a surrogate for total MCs, because MC-LR is reported to be the most toxic MC variant. No authoritative assessments or health-based screening levels, drinking water or otherwise, were identified for NOD. Acute (exposure for a single day) and subchronic (up to 10% of lifetime) health-based screening levels for two cyanotoxin exposure scenarios—contaminated water or cyanobacterial mat/crust consumption—were identified for dogs and cattle. These screening levels were developed for MC congeners: MC-LA, MC-LR, MC-RR, and MC-YR, as well as CYN and ATX-a.

A search of the peer-reviewed scientific literature was conducted to identify available cyanotoxin toxicity studies that could be used as a basis for developing acute (short-term) oral toxicity values (i.e., reference doses (RfDs)) for humans, dogs, cattle, and horses. Full data extraction was performed for two types of priority studies for use in the toxicity factor evaluation: (1) experimental animal, acute, multi-dose studies with oral administration; and (2) case reports of cyanotoxin poisoning in humans and animals. Relevant toxicity data were identified and extracted for MCs, CYN, ATX-a and dhATX-a. Toxicity data identified for NOD were insufficient for use in development of toxicity factors.

The most suitable studies from the literature review were used to develop candidate acute (short-term) RfDs for MC-LR, CYN, ATX-a, and dhATX-a. No-observed-adverse-effect levels (NOAELs), lowest-observed-adverse-effect-levels (LOAELs), or benchmark dose (BMD) modeling of important endpoints from short-term oral toxicity studies were used in RfD development. RfDs based on lethal dose for 50 percent of the population ($LD_{50}$) values were developed for ATX-a and dhATX-a, due to limited available applicable toxicity data. The RfD values derived herein should be considered short-term values and are not meant to be protective of either subchronic or chronic exposures. Acute (short-

term) health-based drinking water screening levels for adults, children, dogs, dairy and beef cattle, and horses were calculated from the respective candidate RfD values. In addition, mat/crust consumption-based screening levels were developed for dogs, dairy and beef cattle, and horses.

# 1 Background

Periodically, TCEQ is asked to address cyanotoxin contamination in drinking and surface water due to harmful algal blooms (HABs) originating from proliferation of cyanobacteria. In the past, these blooms have been linked to illness and death in dogs and livestock (e.g., cattle, horses), as well as contamination of municipal drinking water. While there are no federal maximum contaminant levels (MCLs) for cyanotoxins, the United States Environmental Protection Agency (USEPA) and other agencies have developed regulatory screening levels for various types of cyanotoxins.

ToxStrategies was asked to review the existing health-based screening levels and toxicity factors for cyanotoxins on the Unregulated Contaminant Monitoring Rule 4 (UCMR4) list, as well as for dihydroanatoxin-a, and to develop acute (short-term) toxicity factors and associated health-based screening levels where appropriate and feasible for both drinking water and for consumption of mats/crust by dogs and livestock. The UCMR4 list of cyanotoxins evaluated in this report includes one cyanotoxin group (total microcystins [total MCs]) and nine cyanotoxins: anatoxin-a (ATX-a), cylindrospermopsin (CYN), microcystin-LA (MC-LA), microcystin-LF (MC-LF), microcystin-LR (MC-LR), microcystin-LY (MC-LY), microcystin-RR (MC-RR), microcystin-YR (MC-YR), and nodularin (NOD). At the request of TCEQ, ToxStrategies also evaluated dihydroanatoxin-a (dhATX-a).

# 2 Regulatory Document Review

Existing cyanotoxin health-based screening levels for humans, dogs, cattle, and horses were compiled by reviewing relevant documents produced by federal, state, international, and global regulatory bodies and were identified by searching regulatory websites, as well as ToxPlanet to ensure the identification of all relevant authoritative resources.

## 2.1 Existing Human Health-Based Drinking Water Screening levels

Existing human health-based drinking water screening levels were identified for MCs, CYN, and ATX-a (see Table 1 and Appendix A.1). Table 1 provides summary information for the existing human health-based drinking water screening levels; additional detail is provided in Appendix A.1, In the US, drinking water screening levels have been established by the USEPA and state agencies, including the Minnesota Department of Health, Ohio EPA, and Oregon Health Authority. In addition to the US, the World Health Organization (WHO) and several other countries—including Canada (Health Canada), Australia, New Zealand, Brazil, Uruguay, China, Czech Republic, Denmark, Germany, Italy, Japan, Korea, Netherlands, Norway, Poland, South Africa, Spain, France, and Finland—have established drinking water screening levels for one or more cyanotoxins.

The types of human drinking water screening levels established for MCs, CYN, and ATX-a ranged from "do not drink" drinking water thresholds[1], to short-term or 10-day health advisories, to lifetime drinking water exposure screening levels. Some drinking water screening levels are specific to children or adults, while others are more general. These health-based screening levels across the various government entities were developed using similar toxicity studies for each cyanotoxin. The bases for these screening levels are discussed below. Note that when reporting doses from single dose oral toxicity studies (e.g., gavage) doses are typically expressed as milligram per kilogram (mg/kg) or microgram per kilogram (µg/kg), as opposed to multi-day studies, whereby doses are expressed in microgram per kilogram per day (mg/kg-day) or microgram per kilogram per day (µg/kg-day) (USEPA, 2002).

All MC health-based drinking water screening levels used the congener MC-LR as a surrogate for total MCs, because MC-LR was reported to be the most toxic MC variant. All the existing MC human health drinking water screening levels were based on one of two studies: Heinze (1999) or Fawell et al. (1999a). In Heinze (1999) male rats (N = 5/group) were offered MC-LR in drinking water for 28 days resulting in doses of 0, 50, or 150 µg/kg-day. Increased liver weight, liver lesions in the parenchyma, and increased serum alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) levels were observed in both dose groups, with increased severity of adverse effects in the higher dose group. Degeneration in the hepatocytes was observed as individual cell necrosis, increasing cell volume and increasing mitochondria. Strong activation of Kupffer cells occurred, and increased amounts of periodic acid-Schiff reagent (PAS)-positive substances were measured, suggestive of liver cell damage. Further, lesions included a macroscopic lesion of the liver observed in one animal per dose group -- a dystrophic section of the liver and a hematoma. The lowest-observed-adverse-effect level (LOAEL) was determined to be 50 µg/kg-day based on increased incidence of liver lesions. In Fawell et al. (1999a) mice (N = 15/sex/group) were dosed with MC-LR at 0, 40, 200, or 1000 µg/kg-day MC-LR by oral gavage for 13 weeks. Slight hepatic damage was observed at 200 µg/kg-bw/day based on liver lesions (e.g., pervasive, multifocal hepatocyte degradation in the liver lobule and multifocal, slight chronic inflammation with hemosiderin deposits) and clinical parameters, including increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transaminase (GGT). Because no cyanotoxin-related effects were observed at 40 µg/kg-day, 40 µg/kg-day was determined to be the no-observed-adverse-effect level (NOAEL).

For CYN, all human health drinking water screening levels were based primarily on the study by Humpage and Falconer (2003). In this study, male mice (N = 5 for the high dose group; N= 10/group for other dose groups, N= 12 for controls) were administered 0, 30,

[1] Ohio EPA (2020) has "do not drink" drinking water thresholds for various cyanotoxins (Table 1). The public is advised to not drink water with cyanotoxin levels exceeding the thresholds. Alternative water should be used for drinking, making ice, making infant formula, preparing food, and brushing teeth.

60, 120, or 240 µg/kg-day CYN by oral gavage for 11 weeks. Significant dose-related adverse effects included increased kidney and liver weights, decreased serum bile acid concentrations and increased serum bilirubin levels. The kidney was determined to be the most sensitive target of toxicity, and authors established a NOAEL of 30 µg/kg-day.

Human health drinking water screening levels for ATX-a were based on one of two main studies: Fawell et al. (1999b) or Astrachan et al. (1981). In Fawell et al. (1999b) mice (N= 10/sex/group) were administered 0, 0.098, 0.49, or 2.46 mg/kg-day ATX-a via oral gavage for 28 days. The cause of two deaths, one in each of the mid- and high-dose groups, could not be determined, nor could the authors determine if the deaths were cyanotoxin-related. Specifically, the animals died within 2.5 h of dosing on days 10 and 14 of administration, respectively. Microscopic evaluations were unable to determine cause of death, necropsy evaluation revealed nothing remarkable, and the animals did not show any unusual clinical signs prior to death. Since the authors could not exclude a possible relationship with ATX-a administration, 98 (or 100, rounded up) µg/kg-day was established as the NOAEL, because all surviving animals displayed no adverse effects. Astrachan et al. (1981) administered ATX-a to rats in drinking water at concentrations of 0, 0.5, and 5 ppm for 7 weeks. No changes were observed in serum enzymes (cholinesterase, ALP, glutamic pyruvic transaminase and gamma glutamyl transpeptidase) or tissue histopathology at any dose. The Ohio EPA determined the NOAEL for this study to be equal to 50 µg/kg-bw/day.

Various regulatory agencies used NOAELs or LOAELs from the studies described above, in combination with uncertainty factors, to calculate tolerable daily intakes (TDIs) or reference doses (RfDs) for MC, CYN, or ATX-a. Drinking water intake rates for children or adults were applied to the estimated toxicity factors with or without a relative source contribution (RSC) factor, to develop health-based drinking water screening levels.

**Table 1. Existing human health-based drinking water screening levels for cyanotoxins**

| Cyanotoxin* | Regulatory Agency | Type of Drinking Water Screening level | Description of Screening level | Drinking Water Screening level (µg/L) |
|---|---|---|---|---|
| Microcystins | USEPA (2015a) | 10-Day Health Advisory | Children pre-school age and younger (under 6 years old); applied as total microcystins using microcystin-LR as a surrogate | 0.3 |

| Cyanotoxin* | Regulatory Agency | Type of Drinking Water Screening level | Description of Screening level | Drinking Water Screening level (µg/L) |
|---|---|---|---|---|
| Microcystins | USEPA (2015a) | 10-Day Health Advisory | School-age children (6 years and older); applied as total microcystins using microcystin-LR as a surrogate | 1.6 |
| Microcystins | WHO (2020a) | Provisional Guideline Value; based on lifetime drinking water exposure | Microcystin-LR (free plus cell-bound microcystins) for adults lifetime exposure | 1 |
| Microcystins | WHO (2020a) | Provisional Guideline Value; based on short-term drinking water exposure (2 weeks) | Microcystin-LR (free plus cell-bound microcystins) for adults short-term exposure (up to 2 weeks) | 12 |
| Microcystins | Brazil, Uruguay, China, Czech Republic, Denmark, Germany, Italy, Japan, Korea, Netherlands, Norway, New Zealand, Poland, South Africa, Spain, France, Finland (WHO, 2017) | Drinking Water Guideline | Microcystin-LR (free plus cell-bound microcystins) for adults lifetime exposure | 1 |
| Microcystins | Australia, NHMRC, NRMMC (2011) | Drinking Water Guideline | Total microcystins expressed as microcystin-LR toxicity equivalents for lifetime exposure | 1.3 |
| Microcystins | Health Canada (2017) | Drinking Water Guideline | Total microcystins expressed as microcystin-LR toxicity equivalents; for seasonal exposure (<30 days) | 1.5 |
| Microcystins | Minnesota Department of Health (2015) | Guideline Value | Acute (1-day or less), Short-term (>1 day to 30 days), Subchronic (3- days to 10% of lifetime), and Chronic Non-Cancer Health Based Value | 0.1 |

| Cyanotoxin* | Regulatory Agency | Type of Drinking Water Screening level | Description of Screening level | Drinking Water Screening level (µg/L) |
|---|---|---|---|---|
| Microcystins | Oregon Health Authority (2019) | Drinking Water Guideline | Ages 5 years and younger; up to 10 days | 0.3 |
| Microcystins | Oregon Health Authority (2019) | Drinking Water Guideline | Adults; up to 10 days | 1.6 |
| Microcystins | Ohio EPA (2020) | Drinking Water Threshold | Do Not Drink – children under 6 and sensitive populations; up to 10 days | 0.3 |
| Microcystins | Ohio EPA (2020) | Drinking Water Threshold | Do Not Drink – children 6 and older and adults; up to 10 days | 1.6 |
| Cylindrospermopsin | USEPA (2015b) | 10-Day Health Advisory | Children pre-school age and younger (under 6 years old) | 0.7 |
| Cylindrospermopsin | USEPA (2015b) | 10-Day Health Advisory | School-age children (6 years and older) | 3 |
| Cylindrospermopsin | WHO (2020b) | Provisional lifetime drinking water health-based guidance value | Adult lifetime | 0.7 |
| Cylindrospermopsin | WHO (2020b) | Provisional Guideline Value; based on short-term exposure | Adult short-term (up to 2 weeks) | 3.0 |
| Cylindrospermopsin | Australia (2018) | Health Alert | Due to lack of adequate data, no guideline is set for cylindrospermopsin; however, an initial health alert is estimated | 1 |
| Cylindrospermopsin | New Zealand (2018) | Drinking Water Standard | Provisional maximum acceptable value; lifetime | 1 |
| Cylindrospermopsin | Brazil (2009) | Guideline for Drinking Water Quality (Recommended) | N/A | 15 |
| Cylindrospermopsin | Oregon Health Authority (2019) | Drinking Water Guideline | Ages 5 years and younger; up to 10 days | 0.7 |
| Cylindrospermopsin | Oregon Health Authority (2019) | Drinking Water Guideline | Adults; up to 10 days | 3 |

| Cyanotoxin* | Regulatory Agency | Type of Drinking Water Screening level | Description of Screening level | Drinking Water Screening level (µg/L) |
|---|---|---|---|---|
| Cylindrospermopsin | Ohio EPA (2020) | Drinking Water Threshold | Do Not Drink – children under 6 and sensitive populations; up to 10 days | 0.7 |
| Cylindrospermopsin | Ohio EPA (2020) | Drinking Water Threshold | Do Not Drink – children 6 and older and adults; up to 10 days | 3.0 |
| Anatoxin-a | USEPA (2015c) | No drinking water value | Unable to derive due to lack of data | N/A |
| Anatoxin-a | WHO (2020c) | Provisional short-term drinking water health-based reference value | Adults; up to 2 weeks | 30 |
| Anatoxin-a | New Zealand | Drinking Water Guideline | Provisional maximum acceptable value; lifetime | 6 |
| Anatoxin-a | Minnesota Department of Health (2016) | Risk Assessment Advice | Short-term Non-Cancer Risk Assessment Advice (>1 day to 30 days) | 0.1 |
| Anatoxin-a | Oregon Health Authority (2019) | Drinking Water Guideline | Ages 5 years and younger; up to 10 days | 0.7 |
| Anatoxin-a | Oregon Health Authority (2019) | Drinking Water Guideline | Adults; up to 10 days | 3 |
| Anatoxin-a | Ohio EPA (2020) | Drinking Water Threshold | Do Not Drink – children under 6 and sensitive populations; up to 10 days | 0.3 |
| Anatoxin-a | Ohio EPA (2020) | Drinking Water Threshold | Do Not Drink – children 6 and older and adults; up to 10 days | 1.6 |

*Screening level applies to total cyanotoxins for the class (e.g., total microcystins), unless indicated differently in the Description of Screening level column.

Ohio EPA – Ohio Environmental Protection Agency, N/A – Not available, WHO – World Health Organization, USEPA – United States Environmental Protection Agency

## 2.2 Existing Health-Based Screening Levels for Dogs and Livestock

Acute (exposure for a single day) and subchronic (up to 10% of lifetime) health-based screening levels for two cyanotoxin exposure scenarios—contaminated water or cyanobacterial mat/crust consumption—were identified for dogs and cattle. No existing screening levels were identified for horses. These health-based screening levels were developed for MC congeners (MC-LA, MC-LR, MC-RR, and MC-YR), as well as CYN and ATX-a (see Table 2 and Appendix Table A.2) by the California EPA's Office of Environmental Health Hazard Assessment (OEHHA, 2012). No other health-based screening levels for consumption of water or crust were identified for dogs, cattle, or horses. Table 2 provides summary information for the existing screening levels for dogs and livestock; additional detail is provided in Appendix A.1

For these existing health-based screening levels, reference doses (RfDs) were derived for both species using the same acute or subchronic toxicity study, then applied to both dogs and cattle using species-specific water and crust (dried cyanobacterial scum or mats) consumption factors (OEHHA, 2012). Dog exposure scenarios were estimated for a 20-kg dog and accounted for both drinking and licking water from their coats. In addition, the potential ingestion of crust or mat material was also estimated for dogs. To be most conservative, exposure scenarios for cattle were based on a small breed of dairy cows (e.g., Jersey cows), because their potential exposure to cyanotoxins is greatest due to lower body weight compared to other breeds of cattle. Cattle with lower body weights yield higher calculated intakes of cyanobacteria-contaminated water and crust. Per OEHHA (2012), Jersey cows have lower average body weights (454 kg) relative to large breed dairy cows (e.g., Holsteins [680 kg average body weight]) and beef cattle [635 kg average body weight]). Consumption of cyanobacterial crust on the edge of natural or impounded water bodies, in addition to water consumption, was also considered in cattle exposure estimates. Additional details on dog and livestock exposure scenario estimates are described in Appendix A.2.

Acute health-based screening levels for dogs and cattle for MC congeners LA, LR, RR, and YR were based on the same toxicity study conducted in sheep (Jackson et al., 1984). The oral lethal NOAEL of 1010 mg lyophilized *Microcystis aeruginosa*/kg-bw in sheep administered a single dose of *M. aeruginosa* injected directly into the rumen was used to calculate the RfD for all MC congeners (MC-LA, MC-LR, MC-RR and MC-YR). OEHHA used this NOAEL, in combination with mouse mortality studies conducted by Jackson et al. (1984) and Ellman et al. (1978), to estimate the RfD (OEHHA, 2012). Subchronic health-based screening levels were derived from the 28-day MC-LR drinking water study in rats conducted by Heinz et al. (1999), as described previously in Section 2.1.

CYN acute health-based screening levels for dogs and cattle were calculated using mortality studies conducted in mice as a basis for an acute RfD (Seawright et al., 1999; Shaw et al., 2000, 2001). Seawright et al. (1999) exposed mice via single oral gavage to 4.4–8.3 mg/kg CYN as a suspension of freeze-dried cells and identified the lowest dose

to induce lethality (which was 4.4 mg/kg), within 2-6 days, whereas Shaw et al. (2000) exposed mice to 0, 2, 4, 6, or 8 mg/kg CYN via a single oral administration of sonicated cell extract and found lethality at ≥6 mg/kg. The highest non-lethal dose, 4.0 mg/kg, was determined to be the NOAEL. Subchronic health-based screening levels for CYN were estimated by OEHHA based on Humpage and Falconer's (2003) 11-week gavage study in mice (see Section 2.1).

The 5- and 28-day studies in mice performed by Fawell et al. (1999b; study described in Section 2.1) were used to estimate an acute and subchronic RfD for ATX-a for dogs and cattle. The non-lethal dose of ATX-a of 2.5 mg/kg-day was determined to be the NOAEL. OEHHA applied species-specific uncertainty factors to the NOAELs for each cyanotoxin to estimate RfDs. Water and crust intake rates based on the exposure scenarios described above were then applied by OEHHA to calculate health-based screening levels for dogs and cattle.

**Table 2. Existing dog and livestock regulatory screening levels for cyanotoxins**

| Cyanotoxin | Regulatory Agency | Description of Screening Level | Species* | Screening Level for Water Intake (µg/L) | Screening Level for Crust & Mat Consumption (mg/kg-dry weight) |
|---|---|---|---|---|---|
| Microcystins (Includes microcystins LA, LR, RR, and YR) | OEHHA (2012) | Acute (<24 hrs, exposure for a single day) | Dog | 100 | 0.5 |
| Microcystins (Includes microcystins LA, LR, RR, and YR) | OEHHA (2012) | Acute (<24 hrs, exposure for a single day) | Cattle | 50 | 5 |
| Microcystins (Includes microcystins LA, LR, RR, and YR) | OEHHA (2012) | Subchronic (up to 10% of lifetime) | Dog | 2 | 0.01 |
| Microcystins (Includes microcystins LA, LR, RR, and YR) | OEHHA (2012) | Subchronic (up to 10% of lifetime) | Cattle | 0.9 | 1 |
| Cylindrospermopsin | OEHHA (2012) | Acute (<24 hrs, exposure for a single day) | Dog | 200 | 0.5 |

| | | | | | |
|---|---|---|---|---|---|
| Cylindrospermopsin | OEHHA (2012) | Acute (<24 hrs, exposure for a single day) | Cattle | 60 | 5 |
| Cylindrospermopsin | OEHHA (2012) | Subchronic (up to 10% of lifetime) | Dog | 10 | 0.04 |
| Cylindrospermopsin | OEHHA (2012) | Subchronic (up to 10% of lifetime) | Cattle | 5 | 0.4 |
| Anatoxin-a | OEHHA (2012) | Acute (<24 hrs, exposure for a single day) | Dog | 100 | 0.3 |
| Anatoxin-a | OEHHA (2012) | Acute (<24 hrs, exposure for a single day) | Cattle | 40 | 3 |
| Anatoxin-a | OEHHA (2012) | Subchronic (up to 10% of lifetime) | Dog | 100 | 0.3 |
| Anatoxin-a | OEHHA (2012) | Subchronic (up to 10% of lifetime) | Cattle | 40 | 3 |

* Cattle screening levels based on small breed dairy cow exposure scenario (average body weight of 454 kg). OEHHA - California EPA's Office of Environmental Health Hazard Assessment

# 3 Literature Review

## 3.1 Primary Literature Search

Following the review of the secondary literature as described in Section 2 of this report, a primary literature search was conducted to identify toxicity studies with applicable toxicity information for the 11 cyanotoxins of interest. The literature search was conducted on March 18, 2021, using two bibliographic databases: PubMed and Embase.

For cyanotoxins for which health assessments have been conducted (MC and CYN), the searches were focused on literature published after the health assessments were developed. Therefore, these searches were limited to studies published in 2013 or later, corresponding to the date of searches described in the USEPA evaluations (USEPA, 2015a,b). Literature searches for cyanotoxins ATX-A, dhATX-A, and NOD, which were not covered in prior health assessments, were not restricted by publication date. Cyanotoxin names and synonyms were paired with general toxicity and safety key words, and the search syntax was tailored for each database (Box 1).

## Box 1. Search syntax for cyanotoxins literature searches performed on March 18, 2021, in PubMed and Embase

**Date limited search (PubMed):**

(Cylindrospermopsin OR HSDB 7752 OR "cylindrospermopsin" [Supplementary Concept] OR Microcystins OR microcystin OR Cyanoginosin OR "Microcystins"[Mesh] OR Microcystin-LA OR Microcystis aeruginosa OR "Microcystin LA" OR Microcystin-leucine-alanine OR Microcystin-LF OR "microcystin-LF" [Supplementary Concept] OR Microcystin-LR OR Cyanoginosin LA OR 3-tyrosyl-5-arginine- OR Cyanoginosin YR OR Cyanoginosin-YR OR cyanoginosin LA OR 3-L-tyrosyl-5-L-arginine OR Microcystin-LY OR Microcystin LY OR "microcystin LY" [Supplementary Concept] OR Microcystin-RR OR Cyanoginosin LA OR 3-arginyl-5-arginine- OR "Cyanoginosin RR" OR Cyanoginosin-RR OR Microcystin RR OR Cyanoginosin LA OR 3-L-arginyl-5-L-arginine OR "microcystin RR" [Supplementary Concept] OR Microcystin-YR OR Cyanoginosin YR OR Cyanoginosin-YR OR cyanoginosin LA OR 3-L-tyrosyl-5-L-arginine OR "microcystin YR" [Supplementary Concept]) AND (toxicolog* OR LC50 OR LOEL OR NOEL OR "Toxicity Tests"[MeSH] OR mortality OR (adverse AND (effect* OR outcome* OR endpoint*)) OR (toxicity AND (immun* OR renal OR hematologic* OR nephro* OR hepat* OR endocrin* OR neuro* OR intestin* OR gastrointestin* OR cardiovascular OR pulmonary)) OR immunotox* OR nephrotox* OR hepatotox* OR neurotox* OR cardiotox* OR (toxicity AND (development OR developmental OR reproductive)) OR "Teratogenesis"[MeSH] OR "Reproductive and Urinary Physiological Phenomena"[MeSH] OR neoplastic OR carcinogen* OR carcinoma OR tumor* OR "animal bioassay" OR oncogenic* OR malignant OR malignanc* OR genotoxic* OR genotoxicity OR clastogen* OR mutagen* OR "cytogenetic aberration" OR "chromosome aberrations"[MeSH] OR "DNA damage" OR "DNA fragmentation"[Mesh] OR "Mutagenicity Tests"[MeSH Terms] OR "Chemical and Drug Induced Liver Injury"[MeSH] OR "Chemical and Drug Induced Liver Injury, Chronic"[MeSH])

**Date limited search (Embase):**

(Cylindrospermopsin OR 'cylindrospermopsin'/exp OR Microcystins OR microcystin OR Cyanoginosin OR 'microcystin'/exp OR Microcystin-LA OR 'Microcystis aeruginosa' OR 'Microcystin LA' OR Microcystin-leucine-alanine OR 'microcystin la'/exp OR Microcystin-LF OR 'microcystin lf'/exp OR Microcystin-LR OR 'Cyanoginosin LA' OR 3-tyrosyl-5-arginine- OR 'Cyanoginosin YR' OR Cyanoginosin-YR OR 'cyanoginosin LA' OR 3-L-tyrosyl-5-L-arginine OR 'microcystin lr'/exp OR Microcystin-LY OR 'Microcystin LY' OR 'microcystin LY'/exp OR Microcystin-RR OR 'Cyanoginosin LA' OR 3-arginyl-5-arginine- OR 'Cyanoginosin RR' OR Cyanoginosin-RR OR 'Microcystin RR' OR 'Cyanoginosin LA' OR 3-L-arginyl-5-L-arginine OR 'microcystin RR'/exp OR Microcystin-YR OR 'Cyanoginosin YR' OR Cyanoginosin-YR OR 'cyanoginosin LA' OR 3-L-tyrosyl-5-L-arginine OR 'microcystin YR'/exp) AND (human toxicity OR animal toxicity OR acute toxicity OR mortality OR LD50 OR LC50 OR NOAEL OR LOAEL OR adverse)

**Unlimited search (PubMed):**

(Anatoxin-a OR Anatoxin A OR Anatoxin I OR Antx-A OR BRN 5477454 OR HSDB 7750 OR "anatoxin a" [Supplementary Concept] OR dihydroanatoxin-a OR "dihydro anatoxin-a" OR "dihydro anatoxin a" OR Nodularin OR HSDB 7749 OR "nodularin" [Supplementary Concept]) AND (toxicolog* OR LC50 OR LOEL OR NOEL OR "Toxicity Tests"[MeSH] OR mortality OR (adverse AND (effect* OR outcome* OR endpoint*)) OR (toxicity AND (immun* OR renal OR hematologic* OR nephro* OR hepat* OR endocrin* OR neuro* OR intestin* OR gastrointestin* OR cardiovascular OR pulmonary)) OR immunotox* OR nephrotox* OR hepatotox* OR neurotox* OR cardiotox* OR (toxicity AND (development OR developmental OR reproductive)) OR "Teratogenesis"[MeSH] OR "Reproductive and Urinary Physiological Phenomena"[MeSH] OR neoplastic OR carcinogen* OR carcinoma OR tumor* OR "animal bioassay" OR oncogenic* OR malignant OR malignanc* OR genotoxic* OR genotoxicity OR clastogen* OR mutagen* OR "cytogenetic aberration" OR "chromosome aberrations"[MeSH] OR "DNA damage"

OR “DNA fragmentation”[Mesh] OR “Mutagenicity Tests”[MeSH Terms] OR “Chemical and Drug Induced Liver Injury”[MeSH] OR “Chemical and Drug Induced Liver Injury, Chronic”[MeSH])

**Unlimited search (Embase):**

(Anatoxin-a OR 'Anatoxin A' OR 'Anatoxin I' OR Antx-A OR '(+)-Anatoxin alpha' OR 'anatoxin a'/exp OR dihydroanatoxin-a OR ‘dihydro anatoxin-a’ OR ‘dihydro anatoxin a’ OR Nodularin OR 'nodularin'/exp) AND (toxicity AND (human OR animal OR acute) OR mortality OR LD50 OR LC50 OR NOAEL OR LOAEL OR adverse)

Note: The asterisk (*) is used to allow searches to find words that begin with the letters indicated and end in any form.

All citation results were deduplicated in EndNote (version X9.3.2) and subsequently imported to DistillerSR for screening. A small team of reviewers performed the screening of titles and abstracts, with one reviewer per reference, following piloting and calibration of the screening tool. During screening, included studies were categorized based on authors’ reporting in the title and abstract by:

- Cyanotoxin(s) studied
- Study type (*in vivo*, *in vitro*, review)
- Study model (species)
- Duration (acute, subchronic, or chronic)
- Endpoint (e.g., genotoxicity, neurotoxicity).

Following title and abstract screening, the following types of full-text papers were obtained for review, in order to determine, based on full reporting, whether the study was appropriate for informing an acute health-based screening level:

- Study design: epidemiology, case report, or *in vivo* experimental studies investigating one or more of the 11 cyanotoxins.
- Study species: mammalian (human, canine, livestock, experimental mouse or rat, or other mammals).

Invertebrate (e.g., zebra fish experiments) and *in vitro* studies were maintained in a reference list for potential future review. Full data extraction was performed for two types of priority studies for use in the toxicity factor evaluation: (1) experimental animal, multi-dose studies with oral administration and exposure durations of ≤90 days; and (2) case reports of cyanotoxin poisoning in humans and animals.

## 3.2 Literature Search Results

Findings from these efforts were compiled as a Microsoft Excel workbook, herein referred to as the *Cyanotoxins Literature Workbook* that is provided as Attachment A. The data obtained throughout the literature search and study selection are arranged in Excel worksheets as follows:

- W-1 Full list of initially included papers with categorization data
- W-2 Priority studies study-specific data
- W-3 Case report data
- W-4 Citations excluded during title and abstract review

### *3.2.1 Literature Search and Evidence Identification*

Of the 2,680 unique references screened at the title and abstract level, 270 proceeded to full-text screening for prioritization of studies that could potentially inform the toxicity factor evaluation. Of these, 107 studies were canine, livestock, or human case reports or oral, multi-dose animal experiments with exposure durations of ≤ 90 days, which proceeded to data extraction (**Error! Reference source not found.**).

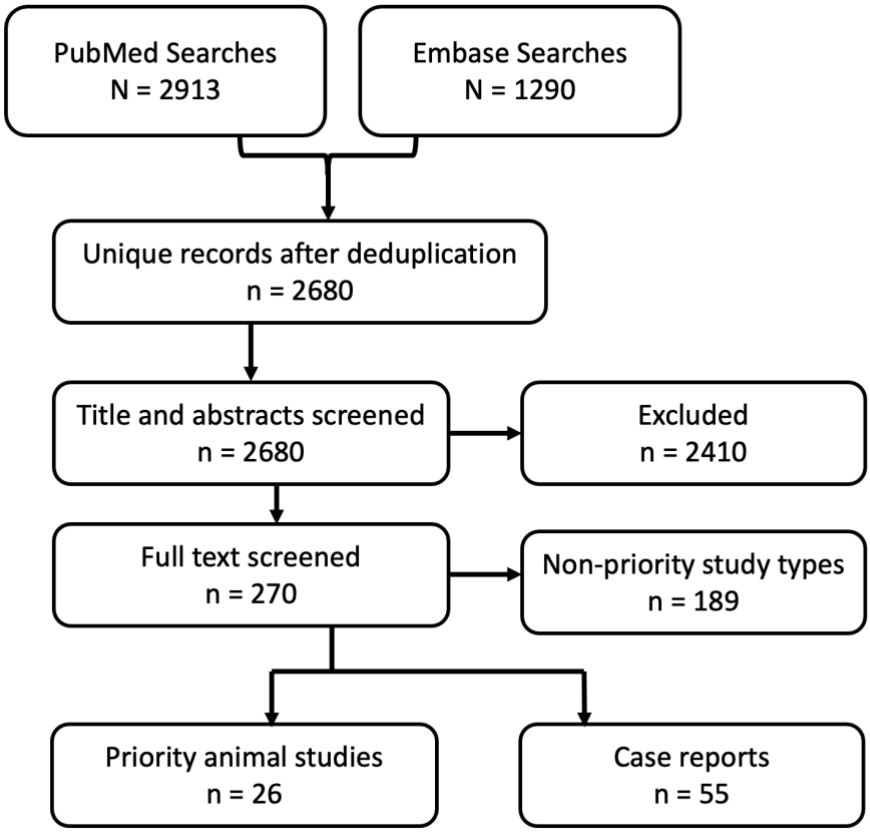


**Figure 1. Flow chart of reviewed citations**

Prioritized studies underwent additional data extraction, which focused on relevant information for the evaluation of existing toxicity factors and potential development of an acute (short-term) health-based screening level. This included study duration, dose levels, and endpoints of acute, multi-dose studies using oral administration, as well as detailed information from canine and livestock case reports. Extracted data are presented in the *Cyanotoxins Literature Workbook* (Attachment A).

### *3.2.2 Data Availability*

MC-LR was the most frequently studied cyanotoxin. Table 3 shows the number of studies evaluating each of the 11 cyanotoxins (or group of cyanotoxins) within the studies that were included during title and abstract review, in the *in vivo* studies that were prioritized for data extraction, and in the case reports detailing human or animal exposure to cyanotoxins in lakes or reservoirs.

**Table 3. Frequency of specific cyanotoxin evaluation**

| Cyanotoxin | Included Studies[a] (N=270*) | Prioritized *In Vivo* Studies[b] (N=26*) | Case Reports (N=55*) |
|---|---|---|---|
| Microcystin-LR | 163 | 20 | 7 |
| Cylindrospermopsin | 38 | 3 | 1 |
| Anatoxin-a | 35 | 1 | 9 |
| Total microcystins | 21 | 2 | 6 |
| Nodularin | 19 | 0 | 3 |
| Microcystin-YR | 9 | 1 | 2 |
| Microcystin-LA | 8 | 1 | 4 |
| Microcystin-RR | 8 | 1 | 2 |
| Dihydroanatoxin-A | 4 | 1 | 4 |
| Microcystin-LY | 2 | 1 | 0 |
| Microcystin-LF | 1 | 1 | 1 |

[a] Included studies from title and abstract screening that were epidemiology, case report, or *in vivo*, mammalian experimental studies investigating one or more of the 11 cyanotoxins.

[b] Prioritized *in vivo* studies were animal studies with oral administration of cyanotoxins for ≤ 90 days.

*As some individual studies investigated more than one cyanotoxin, and not all case reports identified a specific cyanotoxin, numbers in each column do not match the total study N.

### *3.2.3 Priority Studies*

The prioritized studies determined to be most appropriate for the development of short-term health-based screening levels based on duration and endpoint were ultimately relied upon for the evaluation of toxicity factors.

# 4 Modes of Action and Target Tissues

Available information on the potential toxic modes of action for each cyanotoxin are summarized below from the most recent regulatory assessments for cyanotoxin drinking water screening levels conducted by the WHO (2020a,b,c) for MCs, CYN, and ATX-a. No authoritative assessments or drinking water screening levels have been established for nodularin.

## 4.1 Microcystins (MC)

Inhibition of protein phosphatases (PP1, PP2A, and PP5), particularly PP1 and PP2A for a range of MC congeners (Altaner et al., 2019) is considered the molecular initiating event for the toxic responses. This causes a loss of balance between kinase phosphorylation and phosphatase dephosphorylation of cytokeratins, resulting in destabilization of the cytoskeleton and microtubuli (Falconer and Yeung, 1992; Feurstein et al., 2011). Consequently, altered cell function leads to cellular apoptosis and necrosis. Hemorrhage can occur in the liver due to damage of sinusoidal capillaries from loss of cell morphology and cell-to-cell adhesion following acute high doses of microcystins (e.g., 32 µg/kg-day and higher). Alternatively, at lower (<20 µg/kg-day) repeated doses, phosphatase inhibition induces cell proliferation, liver hypertrophy, and tumor promotion activity (Gehringer, 2004).

MC-LR is reported to be the most potent MC variant; however, this conclusion is based on lethal dose for 50 percent of the population ($LD_{50}$) values from intraperitoneal (i.p.) exposure studies. Several studies indicate that most MC variants may have similar protein phosphatase inhibition potency. Pharmacokinetic differences among variants may explain differences in lethal potency, but fewer data are available for MC variant lethality via oral exposure (WHO, 2020a).

## 4.2 Cylindrospermopsin (CYN)

The mode of action for CYN has not been fully determined. However, liver, kidneys, and erythrocytes may be important targets of toxicity. Hepatotoxic effects are caused by the inhibition of protein synthesis (Froscio et al., 2008). Studies investigating the potential for protein synthesis inhibition in the kidneys are not available; however, results of an 11-week oral toxicity study in mice (Humpage and Falconer, 2003) suggest that protein synthesis inhibition also occurs in the kidneys.

## 4.3 Nodularin (NOD)

Due to its similarity in chemical structure to MCs, NODs are anticipated to have a similar mode of action, although there are few mechanistic studies on nodularin toxicity (Buratti et al., 2017). Like MCs, NODs are also hepatotoxic and induce hepatocyte proliferation and tumor promotion (Ohta et al., 1994).

## 4.4 Anatoxins

### *4.4.1 Anatoxin-a (ATX-a)*

ATX-a is a potent neurotoxin that binds with high affinity to the nicotinic acetylcholine receptors (nAChRs) of motor neurons. This nAChR agonist stimulates neurons in the central nervous system causing increased heart rate, blood pressure and muscle cell

contraction. This increase in muscle cell contraction causes fatigue and the eventual paralysis of muscles, which can result in death by asphyxiation when this occurs in respiratory muscles. ATX-a has 100-fold higher affinity for nAChRs than acetylcholine, resulting in a more potent contractive action and muscle overstimulation (Swanson et al., 1986; Fawell et al., 1999b).

### *4.4.2 Dihydroanatoxin-a (dhATX-a)*

The ATX-a congener, dhATX-a, has a structure and mode of action similar to ATX-a. However, a recent study, limited to mortality, suggests that dhATX-a is four-fold more toxic than ATX-a via oral exposure (Puddick et al., 2021).

# 5 Development of Acute (Short-Term) Toxicity Values and Associated Health-based Screening Levels

According to USEPA (2002), one-day health advisories (HAs) are meant to be protective for exposures up to five days and are ideally based on studies of seven days or less. Ten-day HAs are meant to be protective for exposures up to 14 days and are ideally based on studies of 30 days or less. Therefore, where possible, ToxStrategies derived RfD values from studies of 30 days or less. The RfD values herein should be viewed as acute (short-term) RfD values and not meant to be protective of either subchronic or chronic durations.

Standard approaches used by USEPA (2002) and TCEQ (2015) were utilized for the development of the RfDs, particularly for the use of uncertainty factors (UFs). In calculating the RfDs used to develop human screening levels, values of 1, 3, or 10, were applied as appropriate for the interspecies uncertainty factor from animal to human ($UF_A$), intraspecies variability factor ($UF_H$), LOAEL-to-NOAEL uncertainty factor, ($UF_L$), and database uncertainty factor ($UF_D$). When calculating the RfDs used to develop screening levels for dogs, cattle and horses, the $UF_H$ was not applied since this factor is more relevant to humans. No intraspecies variability factor was applied for animals, as there was no empirical basis on which to estimate the variability across types of dogs, cattle and horses pertinent to the establishment of toxicity values. Consistent with USEPA (2002) and TCEQ (2015), default dosimetric adjustment factor [DAFs] were applied to the study point of departure levels to determine human equivalent doses. Default DAFs for converting study-specific doses for adjusting to human equivalent doses include 7 (mouse) and 4 (rat). Appropriate DAFs were derived for determining RfDs for dogs, cattle, and horses using TCEQ (2015) Equation 5-8. These DAFs are included in the Attachment B calculations.

The derivation of candidate RfDs and resulting candidate screening levels is described in the sections below. Tables 4 through 12 list the recommended candidate RfDs, details regarding the studies, and candidate screening levels. Attachment B provides additional detail regarding the calculation of candidate RfDs, candidate screening levels, and

additional values not presented in Tables 4 through 12 (including some candidate RfDs and candidate screening levels not as reliable but included for additional information).

## 5.1 Microcystin-LR

### *5.1.1 Candidate RfD Values for Humans*

Candidate short-term RfD values for MCs were based on toxicity studies for MC-LR ranging in duration from 1 day to 28 days. In Chernoff et al. (2020) BALB/c mice (N= 6 males per dose group and 6-9 females per dose group) were administered a single oral gavage dose of 0, 3, 5, 7 or 9 mg/kg of MC-LR and were necropsied 24 hours after exposure. Endpoints examined included absolute and relative liver weights, serum enzymes (alanine aminotransferase [ALT], aspartate amino transferase [AST], glutamate dehydrogenase [GLDH]), albumin, globulin, total protein, and other clinical chemistry markers. "Moribundity" was used as an endpoint rather than lethality, as the authors indicated the assessment of moribundity (e.g., non-responsiveness to interaction, hunching) provided the maximum amount of information from the animals. The percent of animals considered moribund was increased in male mice at ≥5 mg/kg and in female mice at ≥7 mg/kg. The relative liver weight was among the more sensitive adverse findings and was therefore subjected to benchmark dose (BMD) modeling. Many endpoints (e.g., serum enzymes indicative of liver toxicity [GLDH, AST, ALT (Chernoff et al., 2020)]) appear to have been log transformed prior to reporting summary statistics; these were not modeled, because USEPA BMD guidance recommends against modeling such transformed response data. Instead, USEPA recommends transformation of response data using its Benchmark Dose Software (BMDS) (USEPA, 2012). Note that the variance reported in Chernoff et al. (2020) Table 1 was assumed to be the standard error and therefore was converted to standard deviation prior to modeling. The male relative liver weight data provided a lower BMD than female data—specifically, the $BMDL_{1SD}$ was 2.6 mg/kg-day (2600 mg/kg-day) for males. Due to the short duration of exposure (a single dose with necropsy at 24 hours), allometric scaling was not used to extrapolate from animals to humans (USEPA, 2011). Instead, the $UF_A$)was set to 10 to account for interspecies differences in pharmacokinetics and pharmacodynamics. A default $UF_H$ of 10 was applied to account for sensitive individuals. The total $UF_D$ was set to 10 for deficiencies in the database of short-term studies. Therefore, the candidate RfD based on Chernoff et al. (2020) is 2.6 mg/kg-day (2600 mg/kg-day ÷ 1000).

In Mrdjen et al. (2018) male and female CD-1 mice (N = 10/sex/group) were administered MC-LR via oral gavage at doses of 0, 3000, or 5000 mg/kg-day for seven consecutive days. After one dose of 5000 mg/kg-day, two mice died, and therefore, beginning on the second day the dose was lowered to 4000 mg/kg-day. The overall average daily dose for the high-dose group was 4143 mg/kg-day. Evaluated endpoints included serum enzymes (AST, ALT ALP) and liver histopathology. Histopathologic findings seen in the liver were hypertrophy, degeneration, necrosis, and hemorrhage. BMD modeling of the liver histopathological changes resulted in the lowest $BMDL_{10}$ of

301 µg/kg-day for degeneration in female mice. A default allometric scaling factor of 7 for mice was applied to this value (USEPA, 2002), resulting in a human equivalent dose (HED) of 43 µg/kg-day (301 µg/kg-day ÷ 7). Consistent with TCEQ policy on the allometric scaling of oral studies (pp.144-145 of TCEQ 2015), the $UF_A$ was then set to 1 since allometric scaling was applied. A default $UF_H$ of 10 was applied to account for sensitive individuals. The $UF_D$ was set to 10 for deficiencies in the database of short-term studies. Therefore, the candidate RfD based on Mrdjen et al. (2018) is 0.43 µg/kg-day (43 µg/kg-day ÷ 100).

In Heinze (1999) male rats (N =5/group) bred at the Umweltbundesamt Institute for Water, Soil, and Air Hygiene were offered MC-LR in drinking water for 28 days resulting in doses of 0, 50, or 150 µg/kg-day MC-LR. Endpoints evaluated included serum enzymes (AST, ALP, ALT, and LDH) relative liver weight, and liver histopathology. Histopathologic findings seen in the liver were Kupffer cell activation, degeneration with hemorrhage, and periodic acid Schiff stain (PAS)-positive material. The incidence of these histopathological liver lesions was 100% in the low-dose group, thereby precluding BMD modeling. Based on the liver lesions, 50 µg/kg-day was considered the study LOAEL. A default allometric scaling factor of 4 for rats was applied to this value (USEPA, 2002), resulting in a HED of 12.5 µg/kg-day (50 µg/kg-day ÷ 4). Consistent with TCEQ policy on allometric scaling, the $UF_A$ was set to 1. A default $UF_H$ of 10 was set to account for sensitive individuals. The $UF_L$ was set to 3, based on the absence of a clear NOAEL, and the $UF_D$ was set to 3 for deficiencies in the database. Note that the magnitude of the $UF_D$ used in the derivation of these short-term RfD values decreased as the duration of the studies increased (e.g., it is inherently conservative to use a longer-term study to calculate short-term RfDs, and the consideration of longer-term studies mitigates the database uncertainty associated with acute studies exclusively). Therefore, the candidate RfD based on Heinz (1999) is 0.139 µg/kg-day (12.5 µg/kg-day ÷ 90). Note that the USEPA Health Advisory for MC (USEPA, 2015) uses a RfD of 0.05 µg/kg-day which is also based on liver toxicity as shown by Heinze (1999). Both the candidate and USEPA RfD are derived from the NOAEL of 50 µg/kg-day; however, a DAF of 4 (default allometric scaling factor for rats) and a total UF of 90 ($UF_H$ = 10, $UF_L$ = 3, $UF_D$ = 3) was applied to the candidate RfD. USEPA did not apply a DAF and simply applied a total UF of 1000 to the NOAEL ($UF_A$ = 10, $UF_H$ = 10, $UF_L$ = 3, $UF_D$ = 3 [note the 3s are actually. 3.16 (10^0.5) in USEPA calculations).

In Fawell et al. (1999a) pregnant Crl:Cd-1 mice (N = 26/group) were administered MC-LR via oral gavage at doses of 0, 200, 600, or 2000 µg/kg-day on gestational days 6–15 (GD6–15). On GD18, the dams were necropsied and fetuses were extracted, weighed, and examined for external, visceral, and skeletal abnormalities. Maternal toxicity was observed at 2000 µg/kg-day. Reduced fetal weight and delayed ossification were observed at 2000 µg/kg-day; the study authors considered 600 µg/kg-day as the NOAEL for developmental toxicity. Due to the uncertainties of extrapolating from pregnant dams, allometric scaling was not performed; instead, the $UF_A$ was set to 10 to account for interspecies differences in pharmacokinetics and pharmacodynamics. A default $UF_H$

of 10 was applied to account for sensitive individuals. The $UF_D$ was set to 3 for deficiencies in more detailed reproductive and developmental toxicity studies. A candidate RfD based on Fawell et al. (1999a) would be 2.0 µg/kg-day (600 µg/kg-day ÷ 300). This value was not carried forward, because: (1) a much lower candidate RfD was derived from a study of similar duration (Mrdjen et al., 2018); and (2) Fawell et al. (1999a) did not present any of the developmental toxicity results in tabular or graphical form and is therefore a poorly reported study.

Recent studies have demonstrated developmental effects in BALB/c mice following exposure to low levels of MC-LR in drinking water. Specifically, in Zhang et al. (2017) pregnant BALB/c mice were offered MC-LR at concentrations of 0, 1, 10, and 50 microgram per liter (µg/L) in drinking water from "the 12$^{th}$ day in the embryonic period to the 21$^{st}$ day after birth…the offspring were nursed after natural birth." The study authors estimated that these concentrations equated to doses of 0, 0.1, 1, and 5 µg/kg-day. However, the Methods do not indicate that body weight and water intake were measured, so it is unclear how these dose estimates were determined. The study focused on anogenital distance (AGD), prostate (prostate index; prostatic hyperplasia, fibrosis, necrosis, inflammation; and androgen/ estrogen imbalance), and serum chemistry in male pups at 30 and 90 days after birth. All results were depicted graphically. Body weight was significantly reduced in all dose groups at day 90, albeit without an apparent dose-response. At day 30, body weight was significantly reduced in the intermediate group only. AGD was significantly reduced in all dose groups at day 90, albeit without an apparent dose-response. (AGD was unaffected at day 30). Notably, AGD is often adjusted for body weight( Organisation for Economic Co-operation and Development [OECD 2008a]), which was not done in this study. Body weight was reduced in the mid-dose group at 30 days. Immunostaining for Ki67 (a marker of cell proliferation) was significantly increased in the prostate in the highest dose group at 90 days. Serum testosterone was significantly reduced in all 3 dose groups at 90 days, again without an apparent dose-response. Although mechanistic investigations are beyond the current scope of this project, later phases of masculinization programming are governed by the hypothalamic-pituitary-gonadal (HPG) axis. As such, effects on this axis might lead to reduced testosterone, decreased AGD, and prostate effects. Recent studies indicated that MC-LR can alter the HPG axis of adult male mice at doses of $\geq$ 7.5 µg/kg-day (Xiong et al., 2014), which might explain why the effects reported in Zhang et al. (2017) were observed at 90 days and not at 30 days.

In another reproductive study focusing on male prostate in mice, Han et al. (2019) offered pregnant BALB/c mice MC-LR in drinking water at concentrations of 0, 10, or 50 µg/L from embryonic day (ED)21 to post-natal day (PND) 21. Using the same estimates from Zhang et al. (2017) above, these doses approximately equated to 0, 1, and 5 µg/kg-day. The data are presented graphically without indications of variance or statistical significance but indicate an increase in the percentage of dams having an "abortion" (control = 0%, 10 µg/L = 19%, 50 µg/L = 50%).

Taken together, these newer studies indicated LOAEL values for adverse reproductive and developmental effects at 0.1 or 1 µg/kg-day. Assuming a composite UF of 900 ($UF_A$=10, $UF_H$=10, $UF_L$ = 3, $UF_D$ = 3), the candidate RfD values would be 0.00011 or 0.0011 µg/kg-day. These candidate RfD values are much lower than the RfD values the USEPA (0.05 µg/kg-day) and OEHHA (0.006 µg/kg-day) derived from Heinz et al. (1999) in the development of their HAs for MC-LR. These candidate RfD values based on Zhang et al. (2017) and Han et al. (2019) were not carried forward in our development of health-based screening levels due to concerns about the study quality and consistency with other studies. With regard to study quality, there are deficiencies in the reporting of doses and much of the response data are presented graphically thereby precluding statistical verification and/or dose-response modeling. Several effects also appear to lack a dose-response, calling into question the relationship between exposure and toxicity. Microcystins are considered to be very stable (Fastner and Humpage, 2021); we are unaware of degradation products being of toxicological concern. Given the overall low exposures relative to other MC-LR assays described herein, it seems unlikely that these concentrations have already induced maximal responses between 1-50 ug/L and that the monotonic dose-response lies at lower concentrations. With regard to consistency, we note that Fawell et al. (1999a) did not observe developmental toxicity in mice exposed to much higher doses of MC-LR (200 or 600 µg/kg-day) administered by oral gavage on GD6–15. Although it is not stated in the publication, the Fawell et al. (1999a) study appears to have been conducted at a contract laboratory or government facility and thus may have been conducted in compliance with regulatory guidance such as Good Laboratory Practices. Overall, the reported effects at doses of 0.1 and 1 µg/kg-day following drinking water exposure seem inconsistent with other similar studies where the data are better (i.e., more transparently) reported.

### *5.1.2 Candidate Short-term Health-Based Screening Levels for Humans*

For each of the candidate RfD values (with the exception of the developmental studies by Fawell et al. [1999a], Zhang et al. [2017], and Han et al. [2019]), three candidate screening levels were developed for humans: adult drinking water, child drinking water, and child recreational values. The drinking water values were derived in an analogous fashion to the USEPA Health Advisory for MC (USEPA, 2015). For adults, each candidate RfD was converted to an equivalent water concentration assuming an 80 kg adult consumes 2.5 L of contaminated water per day:

$$HA\ (mg/L) = RfD \times 80\ kg \div 2.5\ L/day$$

For children, water intake data from USEPA's Exposure Factor Handbook (USEPA, 2011) was used to compute a time weighted average 90$^{th}$ percentile for intake of 0.15 L/kg body weight per day for children with the age ranges of 1 to 12 months:

$$HA\ (\mu g/L) = RfD \div 0.15\ L/kg\text{-}day$$

Recreational exposure values were developed using an approach described in OEHHA (2012). OEHHA derived recreational water intake values for different age groups and concluded that children 7 to 10 years of age had the highest ingestion rate while swimming:

$$HA\ (\mu g/L) = RfD \times 30.25\ kg \div 0.25\ L/day$$

Candidate health-based screening levels for drinking water scenarios using the above candidate RfD values are shown in Table 4. Recreational screening levels can be found in the *Cyanotoxin Toxicity Values and Screening Levels Workbook* (Attachment B).

**Table 4. Candidate human health-based drinking water screening levels for microcystin-LR**

| Target Age Group | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Human Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Adult | 2.6 | 1 day | Mouse | 0, 3, 5, 7 or 9 mg/kg | 2600 (BMDL) | 2600 (no DAF) | 1000 ($UF_A$ = 10, $UF_H$ = 10, $UF_D$ = 10) | Relative liver weight | **83.2** | Chernoff et al. (2020) | Drinking water |
| Adult | 0.43 | 7 days | Mouse | 0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day | 301 (BMDL) | 43 (DAF = 7) | 100 ($UF_A$ = 1, $UF_H$ = 10, $UF_D$ = 10) | Liver histopathology (degeneration) | **13.8** | Mrdjen et al. (2018) | Drinking water |
| Adult | 0.139 | 28 days | Rat | 0, 50, or 150 µg/kg-day | 50 (LOAEL) | 12.5 (DAF = 4) | 90 ($UF_A$ = 1, $UF_H$ –= 10, $UF_L$ = 3, $UF_D$ = 3) | Liver lesions (incl. degeneration) | **4.4** | Heinze (1999) | Drinking water |
| *USEPA HA adult** | *0.05* | *28 days* | *Rat* | *0, 50, or 150 µg/kg-day* | *50 (LOAEL)* | *50 (no DAF)* | *1000 ($UF_A$ = 10, $UF_H$ = 10, $UF_L$ = 3, $UF_D$ = 3)* | *Liver toxicity* | ***1.6*** | *Heinze (1999)* | *Drinking water* |
| Child | 2.6 | 1 day | Mouse | 0, 3, 5, 7 or 9 mg/kg | 2600 (BMDL) | 2600 (no DAF) | 1000 ($UF_A$ = 10, $UF_H$ = 10, $UF_D$ = 10) | Relative liver weight | **17** | Chernoff et al. (2020) | Drinking water |

| Target Age Group | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Human Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Child | 0.43 | 7 days | Mouse | 0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day | 301 (BMDL) | 43 (DAF = 7) | 100 ($UF_A$ = 1, $UF_H$ = 10, $UF_D$ = 10) | Liver histopathology (degeneration) | **3** | Mrdjen et al. (2018) | Drinking water |
| Child | 0.139 | 28 days | Rat | 0, 50, or 150 µg/kg-day | 50 (LOAEL) | 12.5 (DAF = 4) | 90 ($UF_A$ = 1, $UF_H$ = 10, $UF_L$= 3, $UF_D$ = 3) | Liver lesions (incl. degeneration) | **0.9** | Heinze (1999) | Drinking water |
| *USEPA HA child** | *0.05* | *28 days* | *Rat* | *0, 50, or 150 µg/kg-day* | *50 (LOAEL)* | *50 (no DAF)* | *1000 ($UF_A$ = 10, $UF_H$ = 10, $UF_L$ = 3, $UF_D$ = 3)* | *Liver toxicity* | ***0.3*** | *Heinze (1999)* | *Drinking water* |

* Regulatory screening levels are indicated with an asterisk and blue, italicized text.

BMDL – benchmark dose level, DAF – dosimetric adjustment factor, HA - health advisory, incl. – including, LOAEL - lowest observed adverse effect level, mg/kg – milligram per kilogram, mg/kg-day – milligram per kilogram per day, OEHHA – California EPA's Office of Environmental Health Hazard Assessment, RfD – reference dose, $UF_A$ - interspecies uncertainty factor from animal to human, $UF_D$ - database uncertainty factor, $UF_H$ - intraspecies variability factor, $UF_L$ - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter, USEPA – US Environmental Protection Agency

### *5.1.3 Candidate RfD Values for Animals*

Candidate RfD values were developed for dogs, horses, and cattle using the studies above with the addition of a one-day study by Jackson et al. (1984) that measured mortality in sheep exposed to microcystin-LR intraruminally. The highest non-lethal dose in that study was 3700 mg/kg. Due to the short duration of exposure (one day), allometric scaling was not used to extrapolate across animal species. Instead, the $UF_A$ was set to 10 to account for interspecies differences in pharmacokinetics and pharmacodynamics. The $UF_D$ was set to 10 for deficiencies in the database of short-term studies. Therefore, the candidate RfD based on Jackson et al. (1984) is 37 mg/kg-day (3700 mg/kg-day ÷ 100). Similarly, the one-day study by Chernoff et al. (2020), see above, results in a candidate RfD of 26 mg/kg-day (2600 mg/kg-day ÷100).

Species-specific candidate RfD values were derived from the seven-day study in mice by Mrdjen et al. (2018) and the 28-day study in rats by Heinz (1999) by taking the point of departure (POD) values described in Section 5.1.1 and allometrically scaling to dogs (20 kg), horses (418 kg) and cattle (590 kg). The body weight for dogs was based on data presented in OEHHA (2012). Body weight for horses was based on USEPA (1988). The body weight values for cattle were based on data from beef cattle (635 kg), small dairy cattle (454 kg) and large dairy cattle (680 kg) contained in OEHHA (2012). The average body weight for the three types of cattle is 590 kg (calculated from OEHHA 2012). Regardless of which cattle body weight was used or whether the cattle body weights were averaged, the allometric scaling factors were comparable, and therefore, a generic RfD was derived for cattle.

The candidate RfD values for dogs, horses, and cattle based on the above toxicity studies are shown in Table 5.

**Table 5. Candidate animal health-based drinking water screening levels for microcystin-LR**

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dogs | 37 | 1 day | Sheep | 0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012]) | 3700 (NOAEL) | 3700 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1 $UF_D$ = 10) | Mortality | **145** | Jackson et al. (1984) | |
| Dogs | 26 | 1 day | Mouse | 0, 3, 5, 7 or 9 mg/kg | 2600 (BMDL | 2600 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | Relative liver weight | **102** | Chernoff et al. (2020) | |
| Dogs | 5.7 | 7 days | Mouse | 0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day | 301 (BMDL) | 57 (DAF = 5.3) | 10 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 10) | Liver histopathology (degeneration) | **22** | Mrdjen et al. (2018) | |
| Dogs | 1.9 | 28 days | Rat | 0, 50, or 150 µg/kg-day | 50 (LOAEL) | 17 (DAF = 3) | 9 ($UF_A$ = 1, $UF_L$ = 3, $UF_D$ = 3) | Liver lesions (incl. degeneration) | **7.3** | Heinze (1999) | |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Dogs** | *37* | *1 day* | *Sheep* | *0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])* | *3700 (NOAEL)* | *3700 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1 $UF_D$ = 10)* | *Mortality* | ***100*** | *Jackson et al. (1984)* | *OEHHA, based on acute RfD* |
| *Dogs** | *0.64* | *28 days* | *Rat* | *0, 50, or 150 µg/kg-day* | *6.4 (BMDL)* | *6.4 (no DAF)* | *10 ($UF_A$ = 3, $UF_D$ = 3)* | *Liver toxicity* | ***2*** | *Heinze (1999)* | *OEHHA, based on subchronic RfD* |
| Dairy cattle | 37 | 1 day | Sheep | 0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012]) | 3700 (NOAEL) | 3700 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1 $UF_D$ = 10) | Mortality | **54** | Jackson et al. (1984) | |
| Dairy cattle | 26 | 1 day | Mouse | 0, 3, 5, 7 or 9 mg/kg | 2600 (BMDL) | 2600 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | Relative liver weight | **38** | Chernoff et al. (2020) | |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dairy cattle | 2.4 | 7 days | Mouse | 0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day | 301 (BMDL) | 24 DAF = 12.4) | 10 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 10) | Liver histopathology (degeneration) | **3.5** | Mrdjen et al. (2018) | |
| Dairy cattle | 0.79 | 28 days | Rat | 0, 50, or 150 µg/kg-day | 50 (LOAEL) | 7 (DAF = 7) | 9 ($UF_A$ = 1, $UF_L$ = 3, $UF_D$ = 3) | Liver lesions (incl. degeneration) | **1.2** | Heinze (1999) | |
| *Dairy cattle** | *37* | *1 day* | *Sheep* | *0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])* | *3700 (NOAEL)* | *3700 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1 $UF_D$ = 10)* | *Mortality* | ***50*** | *Jackson et al. (1984)* | *OEHHA, based on acute RfD* |
| *Dairy cattle** | *0.64* | *28 days* | *Rat* | *0, 50, or 150 µg/kg-day* | *6.4 (BMDL)* | *6.4 (no DAF)* | *10 ($UF_A$ = 3, $UF_D$ = 3)* | *Liver toxicity* | ***0.9*** | *Heinze (1999)* | *OEHHA, based on subchronic RfD* |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Beef cattle | 37 | 1 day | Sheep | 0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012]) | 3700 (NOAEL) | 3700 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | Mortality | **176** | Jackson et al. (1984) | |
| Beef cattle | 26 | 1 day | Mouse | 0, 3, 5, 7 or 9 mg/kg | 2600 (BMDL) | 2600 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | Relative liver weight | **124** | Chernoff et al. (2020) | |
| Beef cattle | 2.4 | 7 days | Mouse | 0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day | 301 (BMDL) | 24 DAF = 12.4) | 10 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 10) | Liver histopat hology (degener ation) | **11.6** | Mrdjen et al. (2018) | |
| Beef cattle | 0.79 | 28 days | Rat | 0, 50, or 150 µg/kg-day | 50 (LOAEL) | 7 (DAF = 7) | 9 ($UF_A$ = 1, $UF_L$ = 3, $UF_D$ = 3) | Liver lesions (incl. degenera tion) | **3.8** | Heinze (1999) | |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Beef cattle** | *37* | *1 day* | *Sheep* | *0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])* | *3700 (NOAEL)* | *3700 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10)* | *Mortality* | ***200*** | *Jackson et al. (1984)* | *OEHHA, based on acute RfD* |
| *Beef cattle** | *0.64* | *28 days* | *Rat* | *0, 50, or 150 µg/kg-day* | *6.4 (BMDL)* | *6.4 (no DAF)* | *10 ($UF_A$ = 3, $U_{FD}$ = 3)* | *Liver toxicity* | ***3*** | *Heinze (1999)* | *OEHHA, based on subchronic RfD* |
| Horses | 37 | 1 day | Sheep | 0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012]) | 3700 (NOAEL) | 3700 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | Mortality | **206** | Jackson et al. (1984) | |
| Horses | 26 | 1 day | Mouse | 0, 3, 5, 7 or 9 mg/kg | 2600 (BMDL) | 2600 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | Relative liver weight | **144** | Chernoff et al. (2020) | |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Horses | 2.6 | 7 days | Mouse | 0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day | 301 (BMDL) | 26 (DAF = 11.8) | 10 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 10) | Liver histopathology (degeneration) | **14** | Mrdjen et al. (2018) | |
| Horses | 0.84 | 28 days | Rat | 0, 50, or 150 µg/kg-day | 50 (LOAEL) | 7.6 (DAF = 6.6) | 9 ($UF_A$ = 1, $UF_L$ = 3, $UF_D$ = 3) | Liver lesions (incl. degeneration) | **5** | Heinze (1999) | |

* Regulatory screening levels are indicated with an asterisk and blue, italicized text.

BMDL – benchmark dose level, DAF – dosimetric adjustment factor, HA - health advisory, incl. – including, LOAEL - lowest observed adverse effect level, MC – microcystin, mg/kg – milligram per kilogram, mg/kg-day – milligram per kilogram per day, NOAEL - no observed adverse effect level, OEHHA – California EPA's Office of Environmental Health Hazard Assessment, RfD – reference dose, $UF_A$ - interspecies uncertainty factor from animal to human, $UF_D$ - database uncertainty factor, $UF_L$ - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter

### *5.1.4 Candidate Short-Term Health-Based Screening Levels for Animals*

#### *5.1.4.1 Candidate Short-Term Health-Based Drinking Water Screening Levels for Animals*

For each of the candidate RfD values above, candidate health-based screening levels were developed for dogs based on a recreational swimming scenario described in OEHHA (2012) and drinking water scenarios for horses, beef cattle, and dairy cattle. For dogs, the exposure scenario was based on ingestion during one hour of exercise/swimming and included exposures from drinking and grooming that result in an estimated ingestion rate of 0.085 L/kg of ingestion per day. According to OEHHA (2012), animals are known to preferentially consume cyanobacteria-contaminated water, therefore OEHHA applied a three-fold factor to this ingestion rate, resulting in 0.255 L/kg body weight per day. The dog HA based on this exposure scenario is as follows:

$$HA_{dog,\ swimming}\ (\mu g/L) = RfD \div 0.255\ L/kg\text{-}day$$

Dairy and beef cattle were estimated to consume water at 0.23 L/kg-day and 0.07 L/kg-day, respectively. Again, OEHHA applied a three-fold factor to this ingestion rate, resulting in 0.69 L/kg-day and 0.21 L/kg-day, respectively:

$$HA_{cattle,\ dairy}\ (\mu g/L) = RfD \div 0.69\ L/kg\text{-}day$$

$$HA_{cattle,\ beef}\ (\mu g/L) = RfD \div 0.21\ L/kg\text{-}day$$

A water intake rate for horses of 0.06 L/kg-day was taken from Freeman et al. (2021). For consistency, we applied a three-fold factor to this ingestion rate, resulting in 0.18 L/kg-day:

$$HA_{horses,}\ (mg/L) = RfD \div 0.18\ L/kg\text{-}day$$

Candidate health-based screening levels for these scenarios using the above candidate RfD values are shown in Table 5.

#### *5.1.4.2 Candidate Short-Term Health-Based Crust/Mat Screening Levels for Animals*

For each of the candidate RfD values above (Section 5.1.3), health-based screening levels for exposure to cyanotoxins in mats and crusts were developed for dogs based on a recreational scenario described in OEHHA (2012) and grazing scenarios for horses, beef cattle, and dairy cattle. For dogs, the exposure scenario was based on the observation that dogs are known to consume an entire day's worth of food/nutrients within just a few minutes. Therefore, it was assumed that a 20-kg dog that consumes ~0.5 kg feed per day (0.025 kg/kg) might consume 0.5 kg of mats and crusts. Again, a

three-fold factor was applied to this ingestion rate, resulting in 0.075 kg mats/kg body weight per day. Therefore, the dog screening level for crust/mat is derived as follows:

$$HA_{dog}\,(mg/kg\ feed) = RfD \div 0.075\ kg/kg\ BW\text{-}day \div 1000\ \mu g/mg$$

Dairy and beef cattle were estimated to consume ~10% of the dietary intake of mats/crusts, which equates to 0.0026 kg/kg body weight per day and 0.0019 kg/kg body weight per day, respectively. OEHHA applied a three-fold factor to this ingestion rate, resulting in 0.0078 kg/kg body weight per day and 0.006 kg/kg body weight per day, respectively. Therefore, the dairy and beef cattle screening levels for crust/mat are calculated as follows:

$$HA_{cattle,\ dairy}\,(mg/kg\ feed) = RfD \div 0.0078\ kg/kg\text{-}day \div 1000\ \mu g/mg$$

$$HA_{cattle,\ beef}\,mg/kg\ feed) = RfD \div 0.006\ kg/kg\text{-}day \div 1000\ \mu g/mg$$

Feed intake in horses was estimated by allometric scaling formulas in USEPA (1998) resulting in 8.6 kg/day (0.065 $\times$ BW^0.7919). Similar to cattle, we assumed that ~10% of the dietary intake would be composed of mats/crusts (i.e., 0.86 kg). Therefore, we estimated an intake rate of 0.0018 kg/kg body weight per day (0.86 kg $\div$ 481 kg). Applying a three-fold factor to this ingestion rate resulted in 0.0054 kg/kg body weight per day. Therefore, the horse screening level for crust/mat is derived as follows:

$$HA_{horse}\,(mg/kg\ feed) = RfD \div 0.0054\ kg/kg\text{-}day \div 1000\ \mu g/mg$$

Candidate health-based screening levels for these scenarios using the above candidate RfD values are shown in Table 6.

**Table 6. Candidate animal mat/crust health-based screening levels for microcystin-LR**

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (mg/kg, dry weight) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dogs | 37 | 1 day | Sheep | 0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012]) | 3700 (NOAEL) | 3700 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | Mortality | **0.5** | Jackson et al. (1984) | |
| Dogs | 26 | 1 day | Mouse | 0, 3, 5, 7 or 9 mg/kg | 2600 (BMDL) | 2600 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | Relative liver weight | **0.35** | Chernoff et al. (2020) | |
| Dogs | 5.7 | 7 days | Mouse | 0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day | 301 (BMDL) | 57 (DAF = 5.3) | 10 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 10) | Liver histopathology (degeneration) | **0.08** | Mrdjen et al. (2018) | |
| Dogs | 1.9 | 28 days | Rat | 0, 50, or 150 µg/kg-day | 50 (LOAEL) | 17 (DAF = 3) | 9 ($UF_A$ = 1, $UF_L$ = 3, $UF_D$ = 3) | Liver lesions (incl. degeneration) | **0.025** | Heinze (1999) | |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (mg/kg, dry weight) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Dogs** | *37* | *1 day* | *Sheep* | *0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])* | *3700 (NOAEL)* | *3700 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10)* | *Mortality* | ***0.5*** | *Jackson et al. (1984)* | *OEHHA, based on acute RfD* |
| *Dogs** | *0.64* | *28 days* | *Rat* | *0, 50, or 150 µg/kg-day* | *6.4 (BMDL)* | *6.4 (no DAF)* | *10 ($UF_A$ = 3, $UF_D$ = 3)* | *Liver toxicity* | ***0.01*** | *Heinze (1999)* | *OEHHA, based on subchronic RfD* |
| Dairy cattle | 37 | 1 day | Sheep | 0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012]) | 3700 (NOAEL) | 3700 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | Mortality | **5** | Jackson et al. (1984) | |
| Dairy cattle | 26 | 1 day | Mouse | 0, 3, 5, 7 or 9 mg/kg | 2600 (BMDL) | 2600 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | Relative liver weight | **3** | Chernoff et al. (2020) | |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (mg/kg, dry weight) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dairy cattle | 2.4 | 7 days | Mouse | 0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day | 301 (BMDL) | 24 DAF = 12.4) | 10 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 10) | Liver histopathology (degeneration) | **0.3** | Mrdjen et al. (2018) | |
| Dairy cattle | 0.79 | 28 days | Rat | 0, 50, or 150 µg/kg-day | 50 (LOAEL) | 7 (DAF = 7) | 9 ($UF_A$ = 1, $UF_L$ = 3, $UF_D$ = 3) | Liver lesions (incl. degeneration) | **0.1** | Heinze (1999) | |
| *Dairy cattle* | *37* | *1 day* | *Sheep* | *0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])* | *3700 (NOAEL)* | *3700 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10)* | *Mortality* | ***5*** | *Jackson et al. (1984)* | *OEHHA, based on acute RfD* |
| *Dairy cattle* | *0.64* | *28 days* | *Rat* | *0, 50, or 150 µg/kg-day* | *6.4 (BMDL)* | *6.4 (no DAF)* | *10 ($UF_A$ = 3, $UF_D$ = 3)* | *Liver toxicity* | ***0.1*** | *Heinze (1999)* | *OEHHA, based on subchronic RfD* |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (mg/kg, dry weight) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Beef cattle | 37 | 1 day | Sheep | 0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012]) | 3700 (NOAEL) | 3700 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | Mortality | **6** | Jackson et al. (1984) | |
| Beef cattle | 26 | 1 day | Mouse | 0, 3, 5, 7 or 9 mg/kg | 2600 (BMDL) | 2600 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | Relative liver weight | **5** | Chernoff et al. (2020) | |
| Beef cattle | 2.4 | 7 days | Mouse | 0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day | 301 (BMDL) | 24 DAF = 12.4) | 10 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 10) | Liver histopathology (degeneration) | **0.4** | Mrdjen et al. (2018) | |
| Beef cattle | 0.79 | 28 days | Rat | 0, 50, or 150 µg/kg-day | 50 (LOAEL) | 7 (DAF = 7) | 9 ($UF_A$ = 1, $UF_L$ = 3, $UF_D$ = 3) | Liver toxicity Liver lesions (incl. degeneration) | **0.1** | Heinze (1999) | |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (mg/kg, dry weight) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Beef cattle** | *37* | *1 day* | *Sheep* | *0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])* | *3700 (NOAEL)* | *3700 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10)* | *Mortality* | ***6*** | *Jackson et al. (1984)* | *OEHHA, based on acute RfD* |
| *Beef cattle** | *0.64* | *28 days* | *Rat* | *0, 50, or 150 µg/kg-day* | *6.4 (BMDL)* | *6.4 (no DAF)* | *10 ($UF_A$ = 3, $UF_D$ = 3)* | *Liver toxicity* | ***0.1*** | *Heinze (1999)* | *OEHHA, based on subchronic RfD* |
| Horses | 37 | 1 day | *Sheep* | *0, 730-1840 mg dry algae/kg (2.7-6.7 mg MC/ kg [OEHHA 2012])* | *3700 (NOAEL)* | *3700 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10)* | Mortality | **6.9** | Jackson et al. (1984) | |
| Horses | 26 | 1 day | Mouse | 0, 3, 5, 7 or 9 mg/kg | 2600 (BMDL) | 2600 (no DAF) | 100 ($UF_A$ = 10 $UF_L$ = 1, $UF_D$ = 10) | Relative liver weight | **4.8** | Chernoff et al. (2020) | |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (mg/kg, dry weight) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Horses | 2.6 | 7 days | Mouse | 0, 3000, or 4000/ 5000 mg/kg-day; average daily dose for high-dose group of 4143 mg/kg-day | 301 (BMDL) | 26 (DAF = 11.8) | 10 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 10) | Liver histopathology (degeneration) | **0.5** | Mrdjen et al. (2018) | |
| Horses | 0.84 | 28 days | Rat | 0, 50, or 150 µg/kg-day | 50 (LOAEL) | 7.6 (DAF = 6.6) | 9 ($UF_A$ = 1, $UF_L$ = 3, $UF_D$ = 3) | Liver toxicity Liver lesions (incl. degeneration) | **0.16** | Heinze (1999) | |

* Regulatory screening levels are indicated with an asterisk and blue, italicized text.

BMDL – benchmark dose level, DAF – dosimetric adjustment factor, HA - health advisory, incl. – including, LOAEL - lowest observed adverse effect level, MC – microcystin, mg/kg – milligram per kilogram, mg/kg-day – milligram per kilogram per day, NOAEL - no observed adverse effect level, OEHHA – California EPA's Office of Environmental Health Hazard Assessment, RfD – reference dose, $UF_A$ - interspecies uncertainty factor from animal to human, $UF_D$ - database uncertainty factor, $UF_L$ - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter

## 5.2 Cylindrospermopsin (CYN)

### *5.2.1 Candidate RfD Values and Short-Term Health-Based Drinking Water Screening Levels for Humans*

Candidate short-term RfD values for CYN were based on a single 11-week study with support from a developmental toxicity study. In Humpage and Falconer (2003) male Swiss Albino mice (N= 10 per group) were administered 0, 30, 60, 120, or 240 µg/kg-day CYN by oral gavage for 11 weeks. At study termination, organs were weighed, clinical chemistry and hematological endpoints were measured, and histological examination was conducted on numerous organs. Urine protein/creatinine levels were significantly decreased at ≥120 µg/kg-day. Serum liver enzymes were not altered significantly. Liver histopathology was altered at 240 µg/kg-day, described as "minor increases in histopathological damage to the liver", but the lesions were not described further. Relative kidney and liver weights were increased significantly at ≥60 µg/kg-day and at 240 µg/kg-day, respectively. The study authors noted that the study NOAEL would be 30 µg/kg-day based on organ weights or 60 µg/kg-day if based on urine protein levels. BMD modeling of the relative kidney weight resulted in a $BMDL_{1SD}$ of 17.9 µg/kg-day. A default allometric scaling factor of 7 for mice was applied to this value (USEPA, 2002), resulting in a HED of 2.6 µg/kg-day (17.9 µg/kg-day ÷ 7), and the $UF_A$ was set to 1. A default $UF_H$ of 10 was applied to account for sensitive individuals. The $UF_D$ was set to 3 for deficiencies in the database. Therefore, the candidate RfD based on Humpage and Falconer (2003) is 0.085 µg/kg-day (2.6 µg/kg-day ÷ 30).

In Sibaldo de Almeida et al. (2013) pregnant Wistar rats (N = 10/group) were given 0, 0.03, 0.3, or 3 µg/kg-day MC-LR by oral gavage on GD1–20. On GD20, the dams were necropsied and fetuses extracted, weighed, and examined for skeletal and visceral malformations. Maternal reproductive organs were evaluated, and the number of fetuses, implantation sites, and resorptions were counted. No adverse effects were observed, and thus, the study NOAEL was 3 µg/kg-day. Due to the uncertainties of extrapolating from pregnant dams, the $UF_A$ was set to 10 to account for interspecies differences in pharmacokinetics and pharmacodynamics. A default $UF_H$ of 10 was applied to account for sensitive individuals. The $UF_D$ was set to 3 for deficiencies in more detailed reproductive and developmental toxicity studies. A candidate RfD based on Sibaldo de Almeida et al. (2013) would be 0.01 µg/kg-day (3 µg/kg-day ÷ 300). This value was not carried forward, because of the "freestanding" NOAEL, with no adverse effects shown in the study with relatively low doses.

The candidate RfD of 0.085 µg/kg-day and the exposure scenarios described in Section 5.1.2 result in the candidate health-based drinking water screening levels shown in Table 7.

**Table 7. Candidate human health-based drinking water screening levels for cylindrospermopsin**

| Target Age Group | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Human Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Adult | 0.085 | 11 weeks | Mouse | 0, 30, 60, 120, or 240 µg/kg-day | 17.9 (BMDL) | 2.6 (DAF = 7) | 30 ($UF_A$ = 1, $UF_H$ = 10, $UF_L$ = 1 $UF_D$ = 3) | Relative Kidney weight | **2.7** | Humpage & Falconer (2003) | Drinking water |
| *USEPA HA adult** | *0.10* | *11 weeks* | *Mouse* | *0, 30, 60, 120, or 240 µg/kg-day* | *30 (NOAEL)* | *30 (no DAF)* | 300 ($UF_A$ = 10, $UF_H$ = 10, $UF_L$ = 1 $UF_D$ = 3) | *Kidney toxicity* | ***3.2*** | *Humpage & Falconer (2003)* | *Drinking water* |
| Child | 0.085 | 11 weeks | Mouse | 0, 30, 60, 120, or 240 µg/kg-day | 17.9 (BMDL) | 2.6 (DAF = 7) | 30 ($UF_A$ = 1, $UF_H$ = 10, $UF_L$ = 1 $UF_D$ = 3) | Relative Kidney weight | **0.6** | Humpage & Falconer (2003) | Drinking water |
| *USEPA HA child** | *0.10* | *11 weeks* | *Mouse* | *0, 30, 60, 120, or 240 µg/kg-day* | *30 (NOAEL)* | *30 (no DAF)* | 300 ($UF_A$ = 10, $UF_H$ = 10, $UF_L$ = 1 $UF_D$ = 3) | *Kidney toxicity* | ***0.7*** | *Humpage & Falconer (2003)* | *Drinking water* |

* Regulatory screening levels are indicated with an asterisk and blue, italicized text.

BMDL – benchmark dose level, DAF – dosimetric adjustment factor, HA - health advisory, NOAEL - no observed adverse effect level, RfD – reference dose, $UF_A$ - interspecies uncertainty factor from animal to human, $UF_D$ - database uncertainty factor, $UF_H$ - intraspecies variability factor, $UF_L$ - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter, USEPA – US Environmental Protection Agency

### *5.2.2 Candidate RfD Values and Short-Term Health-Based Screening Levels for Animals*

Candidate RfD values were developed for dogs, horses, and cattle using Humpage and Falconer (2003) and the same allometric scaling described in Section 5.1.2. In addition, three studies were used to develop candidate RfD values based on mortality. As described in OEHHA (2012), data from Shaw et al. (2000) and Seawright et al. (1999) were used to "derive" a non-lethal dose of CYN. Specifically, Seawright et al. (1999) exposed mice via single oral gavage to 4.4–8.3 mg/kg CYN as a suspension of freeze-dried cells and found the lowest dose to induce lethality within 2-6 days (which was 4.4 mg/kg), whereas Shaw et al. (2000) exposed mice to 0, 2, 4, 6, or 8 mg/kg CYN via a single oral administration of sonicated cell extract and found lethality at ≥6 mg/kg. All mice exposed to the highest dose died within 48 hours; 2 of the 4 mice exposed to the 6 mg/kg dose died within 5 days. No mortality occurred in the remaining dose groups of 0, 1, 2 and 4 mg/kg. Based on both studies combined, OEHHA (2012) selected 4 mg/kg as a non-lethal dose. Due to the short duration of exposure (single dose, mortality observed within 6 days), allometric scaling was not used to extrapolate across animal species. Instead, the $UF_A$ was set to 10 to account for interspecies differences in pharmacokinetics and pharmacodynamics. The $UF_D$ was set to 10 for deficiencies in the database of short-term studies. Therefore, the candidate RfD based on these studies is 40 µg/kg-day (4000 µg/kg-day ÷ 100). For comparison, in Chernoff et al. (2018) male and female CD-1 mice (N= 9-10/sex/group) were administered 0, 75, 150, or 300 µg/kg-day CYN by oral gavage for 90 days. The highest dose was selected as a NOAEL for mortality and extrapolated to dogs, horses, and cattle by allometric scaling (see Section 5.1.3). Due to the conservative use of a 90-day subchronic study to set a short-term (i.e., acute) value, the composite UF was set to 1, resulting in candidate RfD values of 57, 25, and 24 µg/kg-day for dogs, horses, and cattle, respectively. These values are comparable to those based on Shaw et al. (2000) and Seawright et al. (1999). For dogs, the lower candidate RfD of 40 µg/kg-day was carried forward for candidate health-based screening levels, whereas candidate RfD values of 25 and 24 µg/kg-day were carried forward for candidate health-based screening levels for horses and cattle, respectively.

These short-term, candidate RfD values were used to derive candidate health-based screening levels for dogs, cattle, and horses as described above. These values are shown in Tables 8 and 9.

**Table 8. Candidate animal health-based drinking water screening levels for cylindrospermopsin**

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dogs | 40 | 1 day | Mouse | 4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000) | 4000 (NOAEL) | 4000 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1 $UF_D$ = 10) | Mortality | **157** | Shaw et al. (2000) & Seawright et al. (1999) | |
| Dogs | 3.4 | 11 weeks | Mouse | 0, 30, 60, 120, or 240 µg/kg-day | 17.9 (BMDL) | 3.4 (DAF = 5.3) | 1 ($UF_A$ = 1, $UF_L$ = 1 $UF_D$ = 1) | Relative Kidney weight | **13** | Humpage & Falconer (2003) | |
| *Dogs** | *40* | *1 day* | *Mouse* | *4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000)* | *4000 (NOAEL)* | *4000 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ and severity of endpoint = 10)* | *Mortality* | ***200*** | *Shaw et al. (2000) & Seawright et al. (1999)* | *OEHHA, based on acute RfD* |
| *Dogs** | *3.3* | *11 weeks* | *Mouse* | *0, 30, 60, 120, or 240 µg/kg-day* | *33 (BMDL)* | *33 (no DAF)* | *10 ($UF_A$ = 3, $UF_L$ = 1, $UF_D$ = 3)* | *Kidney toxicity* | ***10*** | *Humpage & Falconer (2003)* | *OEHHA, based on subchronic RfD* |
| Dairy cattle | 24 | 13 weeks | Mouse | 0, 75, 150, or 300 µg/kg-day | 300 (NOAEL) | 24 (DAF = 12.4) | *1 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 1)* | Mortality | **35** | Chernoff et al. (2018) | |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dairy cattle | 1.4 | 11 weeks | Mouse | 0, 30, 60, 120, or 240 µg/kg-day | 17.9 (BMDL) | 1.4 (DAF = 12.4) | *1 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 1)* | Relative Kidney weight | **2.1** | Humpage & Falconer (2003) | |
| *Dairy cattle** | *40* | *1 day* | *Mouse* | *4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000)* | *4000 (NOAEL)* | *4000 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1 $UF_D$ = 10)* | *Mortality* | ***60*** | *Shaw et al. (2000) & Seawright et al. (1999)* | *OEHHA, based on acute RfD* |
| *Dairy cattle** | *3.3* | *11 weeks* | *Mouse* | *0, 30, 60, 120, or 240 µg/kg-day* | *33 (BMDL)* | *33 (no DAF)* | *10 ($UF_A$ = 3, $UF_L$ = 1, $UF_D$ = 3)* | *Kidney toxicity* | ***5*** | *Humpage & Falconer (2003)* | *OEHHA, based on subchronic RfD* |
| Beef cattle | 24 | 13 weeks | Mouse | 0, 75, 150, or 300 µg/kg-day | 300 (NOAEL) | 24 (DAF = 12.4) | *1 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 1)* | Mortality | **115** | Chernoff et al. (2018) | |
| Beef cattle | 1.4 | 11 weeks | Mouse | 0, 30, 60, 120, or 240 µg/kg-day | 17.9 (BMDL) | 1.4 (DAF = 12.4) | *1 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 1)* | Relative Kidney weight | **6.9** | Humpage & Falconer (2003) | |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Beef cattle** | *40* | *1 day* | *Mouse* | *4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000)* | *4000 (NOAEL)* | *4000 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1 $UF_D$ = 10)* | *Mortality* | ***200*** | *Shaw et al. (2000) & Seawright et al. (1999)* | *OEHHA, based on acute RfD* |
| *Beef cattle** | *3.3* | *11 weeks* | *Mouse* | *0, 30, 60, 120, or 240 µg/kg-day* | *33 (BMDL)* | *33 (no DAF)* | *10 ($UF_A$ = 3, $UF_L$ = 1, $UF_D$ = 3)* | *Kidney toxicity* | ***20*** | *Humpage & Falconer (2003)* | *OEHHA, based on subchronic RfD* |
| Horses | 25 | 13 weeks | Mouse | 0, 75, 150, or 300 µg/kg-day | 300 (NOAEL) | 25 (DAF = 11.8) | *1 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 1)* | Mortality | **141** | Chernoff et al. (2018) | |
| Horses | 1.5 | 11 weeks | Mouse | 0, 30, 60, 120, or 240 µg/kg-day | 17.9 (BMDL) | 1.5 (DAF = 11.8) | *1 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 1)* | Relative Kidney weight | **8.4** | Humpage & Falconer (2003) | |

* Regulatory screening levels are indicated with an asterisk and blue, italicized text.

BMDL – benchmark dose level, DAF – dosimetric adjustment factor, HA - health advisory, mg/kg – milligram per kilogram, NOAEL - no observed adverse effect level, OEHHA – California EPA's Office of Environmental Health Hazard Assessment, RfD – reference dose, $UF_A$ - interspecies uncertainty factor from animal to human, $UF_D$ - database uncertainty factor, $UF_L$ - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter

**Table 9. Candidate animal mat/crust health-based screening levels for cylindrospermopsin**

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (mg/kg, dry weight) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dogs | 40 | 1 day | Mouse | 4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000) | 4000 (NOAEL) | 4000 (no DAF) | 100 ($UF_A$ = 10, $UF_L$ = 1 $UF_D$ = 10) | Mortality | **0.53** | Shaw et al. (2000) & Seawright et al. (1999) | |
| Dogs | 3.4 | 11 weeks | Mouse | 0, 30, 60, 120, or 240 µg/kg-day | 17.9 (BMDL) | 3.4 (DAF = 5.3) | 1 ($UF_A$ = 1, $UF_L$ = 1 $UF_D$ = 1) | Relative Kidney weight | **0.045** | Humpage & Falconer (2003) | |
| *Dogs** | *40* | *1 day* | *Mouse* | *4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000)* | *4000 (NOAEL)* | *4000 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ and severity of endpoint = 10)* | *Mortality* | ***0.53*** | *Shaw et al. (2000) & Seawright et al. (1999)* | *OEHHA, based on acute RfD* |
| *Dogs** | *3.3* | *11 weeks* | *Mouse* | *0, 30, 60, 120, or 240 µg/kg-day* | *33 (BMDL)* | *33 (no DAF)* | *10 ($UF_A$ = 3, $UF_L$ = 1, $UF_D$ = 3)* | *Kidney toxicity* | ***0.044*** | *Humpage & Falconer (2003)* | *OEHHA, based on subchronic RfD* |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (mg/kg, dry weight) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dairy cattle | 24 | 13 weeks | Mouse | 0, 75, 150, or 300 µg/kg-day | 300 (NOAEL) | 24 (DAF = 12.4) | 1 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 1) | Mortality | **3.1** | Chernoff et al. (2018) | |
| Dairy cattle | 1.4 | 11 weeks | Mouse | 0, 30, 60, 120, or 240 µg/kg-day | 17.9 (BMDL) | 1.4 (DAF = 12.4) | 1 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 1) | Relative Kidney weight | **0.19** | Humpage & Falconer (2003) | |
| *Dairy cattle** | *40* | *1 day* | *Mouse* | *4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000)* | *4000 (NOAEL)* | *4000 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1 $UF_D$ = 10)* | *Mortality* | ***5.1*** | *Shaw et al. (2000) & Seawright et al. (1999)* | *OEHHA, based on acute RfD* |
| *Dairy cattle** | *3.3* | *11 weeks* | *Mouse* | *0, 30, 60, 120, or 240 µg/kg-day* | *33 (BMDL)* | *33 (no DAF)* | *10 ($UF_A$ = 3, $UF_L$ = 1, $UF_D$ = 3)* | *Kidney toxicity* | ***0.42*** | *Humpage & Falconer (2003)* | *OEHHA, based on subchronic RfD* |
| Beef cattle | 24 | 13 weeks | Mouse | 0, 75, 150, or 300 µg/kg-day | 300 (NOAEL) | 24 (DAF = 12.4) | 1 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 1) | Mortality | **4.2** | Chernoff et al. (2018) | |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (mg/kg, dry weight) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Beef cattle | 1.4 | 11 weeks | Mouse | 0, 30, 60, 120, or 240 µg/kg-day | 17.9 (BMDL) | 1.4 (DAF = 12.4) | 1 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 1) | Relative Kidney weight | **0.25** | Humpage & Falconer (2003) | |
| *Beef cattle** | *40* | *1 day* | *Mouse* | *4.4–8.3 mg/kg (Seawright et al. 1999); 0, 2, 4, 6, or 8 mg/kg (Shaw et al. 2000)* | *4000 (NOAEL)* | *4000 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1 $UF_D$ = 10)* | *Mortality* | ***7.0*** | *Shaw et al. (2000) & Seawright et al. (1999)* | *OEHHA, based on acute RfD* |
| *Beef cattle** | *3.3* | *11 weeks* | *Mouse* | *0, 30, 60, 120, or 240 µg/kg-day* | *33 (BMDL)* | *33 (no DAF)* | *10 ($UF_A$ = 3, $UF_L$ = 1, $UF_D$ = 3)* | *Kidney toxicity* | ***0.58*** | *Humpage & Falconer (2003)* | *OEHHA, based on subchronic RfD* |
| Horses | 25 | 13 weeks | Mouse | 0, 75, 150, or 300 µg/kg-day | 300 (NOAEL) | 25 (DAF = 11.8) | 1 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 1) | Mortality | **4.7** | Chernoff et al. (2018) | |
| Horses | 1.5 | 11 weeks | Mouse | 0, 30, 60, 120, or 240 µg/kg-day | 17.9 (BMDL) | 1.5 (DAF = 11.8) | 1 ($UF_A$ = 1, $UF_L$ = 1, $UF_D$ = 1) | Relative Kidney weight | **0.28** | Humpage & Falconer (2003) | |

* Regulatory screening levels are indicated with an asterisk and blue, italicized text.

BMDL – benchmark dose level, DAF – dosimetric adjustment factor, HA - health advisory, mg/kg – milligram per kilogram, NOAEL - no observed adverse effect level, OEHHA – California EPA's Office of Environmental Health Hazard Assessment, RfD – reference dose, $UF_A$ - interspecies uncertainty factor from animal to human, $UF_D$ - database uncertainty factor, $UF_L$ - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter

## 5.3 Nodularins

### *5.3.1 Candidate RfD Values and Short-Term Health-Based Screening Levels for Humans and Animals*

Review of the limited number of studies identified in the literature for NODs, indicated that there were no priority studies (i.e., experimental animal, acute, multi-dose studies with oral administration, or case reports) available for development of candidate RfDs. As a result, candidate RfD and candidate short-term health-based screening levels could not be developed. Until more applicable toxicity data become available for RfD and health-based screening level development, ToxStrategies suggests using candidate human and animal screening levels developed for MCs as surrogates for NODs. MCs and NODs have similar chemical structures and are both hepatoxic, consequently it is anticipated that NODs also share a similar mode of action to MCs (Buratti et al., 2017; Ohta et al., 1994).

## 5.4 Anatoxins

### *5.4.1 Candidate RfD Values and Short-Term Health-Based Screening Levels for Humans and Animals*

Candidate short-term RfD values for ATX-a and dhATX were derived from acute one-dose exposures. Specifically, Puddick et al. (2021) determined the acute $LD_{50}$ values in female Swiss albino mice using an OECD (2008b) "up and down procedure." Mice were administered ATX-a or dhATX via i.p. injection, oral gavage, or admixed in feed. ATX-a was determined to be more potent than dhATX by i.p. injection, whereas dhATX was more potent than ATX-a following oral exposure (gavage or offering in feed). The $LD_{50}$ values for ATX-a and dhATX from gavage exposure were two- to three-fold lower than by feeding. Due to the increased sensitivity of the oral route and the primary concern from water intake, $LD_{50}$ values from oral gavage were selected as the basis for the candidate RfDs. Applying a 10,000-fold composite UF ($UF_A$ = 10, $UF_H$ = 10, $UF_L$ = 10, $UF_D$ = 10) to the $LD_{50}$ for ATX-a resulted in a candidate RfD for humans of 1 µg/kg-day (10,600 µg/kg ÷ 10,000). Applying the same 10,000-fold composite UF to the $LD_{50}$ for dhATX resulted in a candidate RfD for humans of 0.25 µg/kg-day (2500 µg/kg ÷ 10,000). For dogs, cattle and horses, the candidate RfDs based on the same $LD_{50}$ values are 10-fold higher (11 µg/kg-day for ATX-a, 2.5 µg/kg-day for dhATX), as there was no $UF_H$ used in the calculation. These short-term candidate RfD values were used to derive candidate health-based screening levels for adults and children. The short-term candidate RfD values for dogs, cattle, and horses were used to derive candidate health-based screening levels for these species. These candidate values are shown in Tables 10–12.

**Table 10. Candidate human health-based drinking water screening levels for anatoxin-a and dihydroanatoxin-a**

| Target Age Group | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Human Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Adult | 0.25 | 1 day (dhATX-a) | Mouse | Range; using OECD (2008) "up and down" procedure | 2500 ($LD_{50}$) | 2500 (no DAF) | 10,000 ($UF_A$ = 10, $UF_H$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **8** | Puddick et al. (2021) | Drinking water |
| Adult | 1.1 | 1 day (ATX-a) | Mouse | Range; using OECD (2008) "up and down" procedure | 10,600 ($LD_{50}$) | 10,600 (no DAF) | 10,000 ($UF_A$ = 10, $UF_H$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **34** | Puddick et al. (2021) | Drinking water |
| *USEPA HA adult** | *NA* | *NA* | *NA* | *NA* | *NA* | *NA* | *NA* | *NA* | ***No Value*** | *NA* | *Drinking water* |
| Child | 0.25 | 1 day (dhATX-a) | Mouse | Range; using OECD (2008) "up and down" procedure | 2500 ($LD_{50}$) | 2500 (no DAF) | 10,000 ($UF_A$ = 10, $UF_H$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **1.7** | Puddick et al. (2021) | Drinking water |
| Child | 1.1 | 1 day (ATX-a) | Mouse | Range; using OECD (2008) "up and down" procedure | 10,600 ($LD_{50}$) | 10,600 (no DAF) | 10,000 ($UF_A$ = 10, $UF_H$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **7** | Puddick et al. (2021) | Drinking water |
| *USEPA HA child** | *NA* | *NA* | *NA* | *NA* | *NA* | *NA* | *NA* | *NA* | ***No Value*** | *NA* | *Drinking water* |

* Regulatory screening levels are indicated with an asterisk and blue, italicized text .

ATX-a anatoxin-a, BMDL – benchmark dose level, DAF – dosimetric adjustment factor, dhATX-a – dihydroanatoxin-a , HA – health advisory, $LD_{50}$ – lethal dose for 50 percent of the population, NA – not applicable, OECD – Organisation for Economic Co-operation and Development, RfD – reference dose, $UF_A$ – interspecies uncertainty factor from animal to human, $UF_D$ – database uncertainty factor, $UF_H$ – intraspecies variability factor, $UF_L$ – LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter, USEPA – US Environmental Protection Agency

**Table 11. Candidate animal health-based drinking water screening levels for anatoxin-a and dihydroanatoxin-a**

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dogs | 2.5 | 1 day (dhATX-a) | Mouse | Range; using OECD (2008) "up and down" procedure | 2500 ($LD_{50}$) | 2500 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **10** | Puddick et al. (2021) | |
| Dogs | 11 | 1 day (ATX-a) | Mouse | Range; using OECD (2008) "up and down" procedure | 10,600 ($LD_{50}$) | 10,600 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **42** | Puddick et al. (2021) | |
| *Dogs** | *25* | *5 day (ATX-a)* | *Mouse* | *1.2 – 12.3^ mg/kg-day* | *2500^ (NOAEL)* | *2500 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10)* | *Mortality* | ***98*** | *Fawell (1999b)* | *OEHHA, acute and subchronic* |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dairy cattle | 2.5 | 1 day (dhATX-a) | Mouse | Range; using OECD (2008) "up and down" procedure | 2500 ($LD_{50}$) | 2500 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **3.6** | Puddick et al. (2021) | |
| Dairy cattle | 11 | 1 day (ATX-a) | Mouse | Range; using OECD (2008) "up and down" procedure | 10,600 ($LD_{50}$) | 10,600 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **15** | Puddick et al. (2021) | |
| *Dairy cattle** | *25* | *5 day (ATX-a)* | *Mouse* | *1.2 – 12.3^ mg/kg-day* | *2500^ (NOAEL)* | *2500 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10)* | *Mortality* | ***36*** | *Fawell (1999b)* | *OEHHA, acute and subchronic* |
| Beef cattle | 2.5 | 1 day (dhATX-a) | Mouse | Range; using OECD (2008) "up and down" procedure | 2500 ($LD_{50}$) | 2500 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **12** | Puddick et al. (2021) | |
| Beef cattle | 11 | 1 day (ATX-a) | Mouse | Range; using OECD (2008) "up and down" procedure | 10,600 ($LD_{50}$) | 10,600 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **50** | Puddick et al. (2021) | |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (µg/L) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Beef cattle** | *25* | *5 day (ATX-a)* | *Mouse* | *1.2 - 12.3^ mg/kg-day* | *2500^ (NOAEL)* | *2500 (no DAF)* | *100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10)* | *Mortality* | ***119*** | *Fawell (1999b)* | *OEHHA, acute and subchronic* |
| Horses | 2.5 | 1 day (dhATX-a) | Mouse | Range; using OECD (2008) "up and down" procedure | 2500 ($LD_{50}$) | 2500 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **14** | Puddick et al. (2021) | |
| Horses | 11 | 1 day (ATX-a) | Mouse | Range; using OECD (2008) "up and down" procedure | 10,600 ($LD_{50}$) | 10,600 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **59** | Puddick et al. (2021) | |

* Regulatory screening levels are indicated with an asterisk and blue, italicized text.

^Doses are listed as reported by OEHHA (2012). However, doses in the reference study (Fawell 199b) are indicated as 1.5, 3, 7.5 or 15 mg/kg-day, with 3 mg/kg-day as the maximum tolerated dose.

ATX-a anatoxin-a, DAF – dosimetric adjustment factor, dhATX-a - dihydroanatoxin-a , $LD_{50}$ - lethal dose for 50 percent of the population, mg/kg-day – milligram per kilogram per day, NOAEL – no observed adverse effect level, RfD – reference dose, OECD - Organisation for Economic Co-operation and Development, $UF_A$ - interspecies uncertainty factor from animal to human, $UF_D$ - database uncertainty factor, $UF_L$ - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter

**Table 12. Candidate animal mat/crust health-based screening levels for anatoxin-a and dihydroanatoxin-a**

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (mg/kg, dry weight) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dogs | 2.5 | 1 day (dhATX) | Mouse | Range; using OECD (2008) "up and down" procedure | 2500 ($LD_{50}$) | 2500 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **0.033** | Puddick et al. (2021) | |
| Dogs | 11 | 1 day (ATX) | Mouse | Range; using OECD (2008) "up and down" procedure | 10,600 ($LD_{50}$) | 10,600 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **0.14** | Puddick et al. (2021) | |
| *Dogs** | *25* | *5 day (ATX)* | *Mouse* | *1.2 - 12.3^ mg/kg-day* | *2500^ (NOAEL)* | *2500 (no DAF)* | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | *Mortality* | ***0.33*** | *Fawell (1999b)* | *OEHHA, acute and subchronic* |
| Dairy cattle | 2.5 | 1 day (dhATX) | Mouse | Range; using OECD (2008) "up and down" procedure | 2500 ($LD_{50}$) | 2500 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **0.32** | Puddick et al. (2021) | |
| Dairy cattle | 11 | 1 day (ATX) | Mouse | Range; using OECD (2008) "up and down" procedure | 10,600 ($LD_{50}$) | 10,600 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **1.4** | Puddick et al. (2021) | |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (mg/kg, dry weight) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Dairy cattle** | *25* | *5 day (ATX)* | *Mouse* | *1.2 - 12.3^ mg/kg-day* | *250^0 (NOAEL)* | *2500 (no DAF)* | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | *Mortality* | ***3.2*** | *Fawell (1999b)* | *OEHHA, acute and subchronic* |
| Beef cattle | 2.5 | 1 day (dhATX) | Mouse | Range; using OECD (2008) "up and down" procedure | 2500 ($LD_{50}$) | 2500 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **0.44** | Puddick et al. (2021) | |
| Beef cattle | 11 | 1 day (ATX) | Mouse | Range; using OECD (2008) "up and down" procedure | 10,600 ($LD_{50}$) | 10,600 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **1.9** | Puddick et al. (2021) | |
| *Beef cattle** | *25* | *5 day (ATX)* | *Mouse* | *1.2 - 12.3^ mg/kg-day* | *2500^ (NOAEL)* | *2500 (no DAF)* | 100 ($UF_A$ = 10, $UF_L$ = 1, $UF_D$ = 10) | *Mortality* | ***4.4*** | *Fawell (1999b)* | *OEHHA, acute and subchronic* |
| Horses | 2.5 | 1 day (dhATX) | Mouse | Range; using OECD (2008) "up and down" procedure | 2500 ($LD_{50}$) | 2500 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **0.5** | Puddick et al. (2021) | |

| Target Species | Candidate RfD (µg/kg-day) | Study Duration | Study Species | Administered Doses | Point of Departure (µg/kg-day) | Species Equivalent Dose (µg/kg-day) | Uncertainty Factors | Endpoint | Candidate Screening Level (mg/kg, dry weight) | Study | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Horses | 11 | 1 day (ATX) | Mouse | Range; using OECD (2008) "up and down" procedure | 10,600 ($LD_{50}$) | 10,600 (no DAF) | 1000 ($UF_A$ = 10, $UF_L$ = 10, $UF_D$ = 10) | Mortality | **2.0** | Puddick et al. (2021) | |

* Regulatory screening levels are indicated with an asterisk and blue, italicized text .

^Doses are listed as reported by OEHHA (2012). However, doses in the reference study (Fawell 199b) are indicated as 1.5, 3, 7.5 or 15 mg/kg-day, with 3 mg/kg-day as the maximum tolerated dose.

ATX-a anatoxin-a, DAF – dosimetric adjustment factor, dhATX-a - dihydroanatoxin-a , $LD_{50}$ - lethal dose for 50 percent of the population, mg/kg-day – milligram per kilogram per day, NOAEL – no observed adverse effect level, RfD – reference dose, OECD - Organisation for Economic Co-operation and Development, $UF_A$ - interspecies uncertainty factor from animal to human, $UF_D$ - database uncertainty factor, $UF_L$ - LOAEL-to-NOAEL uncertainty factor, µg/kg-day – microgram per kilogram day, µg/L – microgram per liter

Because the TCEQ has guidance for developing chronic RfD values from $LD_{50}$ values for chemicals with limited toxicity data, we applied that guidance to the $LD_{50}$ values above in order to compare hypothetical chronic RfD values to the short-term candidate RfD values. Specifically, each $LD_{50}$ was multiplied by 6.7E-06, resulting in chronic RfD values of 0.07 and 0.02 µg/kg-day for ATX-a and dhATX, respectively. Each of these chronic RfD values was 14-fold lower than its corresponding candidate short-term RfD. Notably, 14-fold is similar to the default 10-fold UF typically used in risk assessment to extrapolate from subchronic to chronic values. Stated differently, one could have developed chronic RfD values for ATX-a and dhATX based on their $LD_{50}$ values and then multiplied them by 10 to estimate shorter-term candidate toxicity values. Overall, these comparisons provide some assurance that the short-term candidate RfD values are reasonable estimates for these data-poor compounds.

Although OEHHA developed RfDs for ATX based on repeat-dose studies by Fawell et al. (1999b) and Astrachan et al. (1980), these studies were deemed unreliable in the present case. The USEPA reached similar conclusions about these two studies (USEPA, 2015c). In Fawell et al. (1999b) male and female Crl:CD-1 mice (N = 10/sex/group) were administered doses of 0, 0.098, 0.49, and 2.46 µg/kg-day ATX-a by oral gavage for 28 days. Three deaths occurred in the study: one female in the high-dose group (day 14), one male in the mid-dose group (day 10), and one male in the low-dose group. The two deaths in the higher dose group occurred within 2.5 hours of dosing, and no cause of death was determined at necropsy. The male in the low-dose group was euthanized after showing signs of being attacked by cage mates. The study authors called 0.098 µg/kg-day the study NOAEL but acknowledged that the "true NOAEL" might be 2.46 µg/kg-day. In Fawell et al. (1999b) pregnant mice (N= unspecified) were administered these same doses on GD5–15 and the dams were necropsied on GD18. The numbers of live and dead implantations were recorded, and the fetuses were weighed and examined for external abnormalities. The authors stated that the NOAEL for the study was 2.6 µg/kg-day; however, no data were shown. A composite UF of 300 was derived based on $UF_A$ = 10, $UF_H$ = 10, $UF_D$ = 3. A candidate RfD for humans based on Fawell et al. (1999b) would be 0.009 µg/kg-day (2.6 µg/kg-day ÷ 300). This value was not carried forward due to: (1) uncertainty related to the early deaths; (2) the limited data reporting; and (3) the limited utility of a "freestanding" NOAEL.

In Astrachan and colleagues (1980, 1981), Sprague Dawley rats (20 females per treatment group; unspecified number in control group) were offered ATX-a in drinking water at concentrations of 0, 0.51, and 5.1 ppm for seven weeks, which the study authors estimated was equivalent to a dose of 0.5 mg/kg-day for the high- dose group. Therefore, we assumed a dose of 0.05 mg/kg-day for the low-dose group. Red and white blood cells were counted and selected clinical chemistry parameters were measured; additional details were not provided by the authors. The methods stated that all animals were "heart-bled" under anesthesia on days 0, 7, 14, 27, 41, and 56. It was not clear whether these animals were terminated at each time point, nor is such information contained in the single figure and there were no tabular data in the Results section. The

study authors concluded that there were no “apparent differences between treated and control groups.” Consistent with conclusions in USEPA (2015c), this study is too poorly reported to be of use in risk assessment.

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APPENDIX A

# Regulatory Drinking Water Screening Levels for Cyanotoxins

## Table A.1. Regulatory human drinking water screening levels for cyanotoxins.

| Regulatory Screening Level | | | | Study Screening Level is Based Upon | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Regulatory Agency | Type of Drinking Water Screening Level | Description of Screening Level | Drinking Water Screening Level (µg/L) | Endpoint | Model | Administration Method | Study Duration | POD | Health-Based Toxicity Value and Screening Level Calculation | References |
| US EPA. 2015. Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins. EPA- 820R15100. | 10-Day Health Advisory | Children pre-school age and younger (under 6 years old) Applied as total microcystins using microcystin-LR as a surrogate | 0.3 | Liver lesions | Male rats | Microcystin-LR in drinking water | 28 days | LOAEL = 50 µg/kg/day | LOAEL/1,000 = RfD; RfD = 0.05 µg/kg-d; (0.05/0.15 L/kg/day; no RSC)=Health Advisory | Heinze, R. 1999. Toxicity of the cyanobacterial toxin microcystin-LR to rats after 28 days intake with the drinking water. Environ. Toxicol., 14(1): 57–60. |
| | | School-age children (6 years and older) Applied as total microcystins using microcystin-LR as a surrogate | 1.6 | | | | | | LOAEL/1,000 = RfD; RfD = 0.05 µg/kg-d; (0.05/0.03 L/kg/day; no RSC)=Health Advisory | |
| WHO. 2020. Cyanobacterial toxins: microcystins. Background document for development of WHO Guidelines for drinking-water quality and Guidelines for safe recreational water environments. WHO/HEP/ECH/WSH/2020.6. | Provisional Guideline Value; based on life-time drinking water exposure | Microcystin-LR (free plus cell-bound microcystins) for adults lifetime exposure | 1 | Liver histopathology and serum enzyme levels | Mice | Microcystin-LR via oral gavage | 13 weeks | NOAEL = 40 µg/kg/day | NOAEL/1,000 = TDI; TDI = 0.04 µg/kg; (0.04 µg/kd-d*60 kg*0.8 RSC)/ (2 L/d)=Guideline value | Fawell JK, Mitchell RE, Everett DJ, Hill RE. 1999. The toxicity of cyanobacterial toxins in the mouse: I. Microcystin-LR. Hum Exper Toxicol. 18:162–7. |
| | Provisional Guideline Value; based on short-term drinking water exposure | Microcystin-LR (free plus cell-bound microcystins) for adults short-term exposure | 12 | | | | | | No toxicity value; (40 µg/kd-d*60 kg*1 RSC) / (100*2 L/d)=Guideline value | |
| Brazil, Uruguay, China, Czech Republic, Denmark, Germany, Italy, Japan, Korea, Netherlands, Norway, New Zealand, Poland, South Africa, Spain, France, Finland | Drinking Water Guideline | Microcystin-LR (free plus cell-bound microcystins) for adults lifetime exposure | 1 | Liver histopathology and serum enzyme levels | Mice | Microcystin-LR via oral gavage | 13 weeks | NOAEL = 40 µg/kg/day | NOAEL/1,000 = TDI; TDI = 0.04 µg/kg; (0.04 µg/kd-d*60 kg*0.8 RSC)/ (2 L/d)=Guideline value | Based on the World Health Organization (WHO) Provisional Guideline Value of 1µgL for drinking water (WHO, 2017)<br>Fawell JK, James CP, James HA. 1994. Toxins from Blue-Green Algae: Toxicological assessment of Microcystin-LR and a method for its determination in water. pp 1-46. Water Research Centre, Medmenham, UK. |
| Australia. 2011. Australian Drinking Water Guidelines 6 (NHMRC, NRMMC) | Drinking Water Guideline | Total microcystins expressed as microcystin-LR toxicity equivalents | 1.3 | Liver histopathology and serum enzyme levels | Mice | Microcystin-LR via oral gavage | 13 weeks | NOAEL = 40 µg/kg/day | No toxicity value; (40 µg/kd-d*70 kg*0.9 RSC)/ (1,000*2 L/d)= Guideline value | Fawell JK, James CP, James HA. 1994. Toxins from Blue-Green Algae: Toxicological assessment of Microcystin-LR and a method for its determination in water. pp 1-46. Water Research Centre, Medmenham, UK. |
| Health Canada. 2017. Guidelines for Canadian Drinking Water Quality: Supporting Documentation Cyanobacterial Toxins-Microcystin-LR. | Drinking Water Guideline | Total microcystins expressed as microcystin-LR toxicity equivalents | 1.5 | Liver lesions | Male rats | Microcystin-LR in drinking water | 28 days | LOAEL = 50 µg/kg/day | LOAEL/ 900 = TDI; TDI = 0.056 µg/kg/day; (0.056 µg/kd*70 kg*0.8 RSC)/ (1.5 L/day)= Guideline value | Heinze, R. 1999. Toxicity of the cyanobacterial toxin microcystin-LR to rats after 28 days intake with the drinking water. Environ. Toxicol., 14(1): 57–60. |
| Minnesota Department of Health. 2015. Toxicological Summary for Microcystin-LR. | Guideline Value | Short-term Non-Cancer Health Based Value | 0.1 | Liver lesions | Male rats | Microcystin-LR in drinking water | 28 days | LOAEL = 50 µg/kg/day | 0.05 mg/kg/day*0.24 = 0.012 mg/kg/day = HED; HED/300 = RfD RfD = 0.04 µg/kg/day; (0.04 µg/kg/day*0.8 RSC)/ (0.289 L/kg/day)=Guideline value | Heinze, R. 1999. Toxicity of the cyanobacterial toxin microcystin-LR to rats after 28 days intake with the drinking water. Environ. Toxicol., 14(1): 57–60. |
| Oregon Health Authority. 2019. Public Health Advisory Guidelines, Harmful Algae Blooms in Freshwater Bodies. | Drinking Water Guideline | Ages 5 years and younger | 0.3 | Liver lesions | Male rats | Microcystin-LR in drinking water | 28 days | LOAEL = 50 µg/kg/day | LOAEL/1,000 = RfD; RfD = 0.05 µg/kg-d; (0.05/0.15 L/kg/day; no RSC)=Guideline value | Heinze, R. 1999. Toxicity of the cyanobacterial toxin microcystin-LR to rats after 28 days intake with the drinking water. Environ. Toxicol., 14(1): 57–60. |
| | | Adults | 1.6 | | | | | | LOAEL/1,000 = RfD; RfD = 0.05 µg/kg-d; (0.05/0.03 L/kg/day; no RSC)=Guideline value | |
| Ohio EPA. 2020. Public Water System Harmful Algal Bloom Response Strategy. | Drinking Water Threshold | Do Not Drink –children under 6 and sensitive populations | 0.3 | Liver lesions | Male rats | Microcystin-LR in drinking water | 28 days | LOAEL = 50 µg/kg/day | LOAEL/1,000 = RfD; RfD = 0.05 µg/kg-d; (0.05/0.15 L/kg/day; no RSC)=Threshold value | Heinze, R. 1999. Toxicity of the cyanobacterial toxin microcystin-LR to rats after 28 days intake with the drinking water. Environ. Toxicol., 14(1): 57–60. |
| | | Do Not Drink –children 6 and older and adults | 1.6 | | | | | | LOAEL/1,000 = RfD; RfD = 0.05 µg/kg-d; (0.05/0.03 L/kg/day; no RSC)=Threshold value | |

## Table A.1 (continued). Regulatory human drinking water guidelines for cyanotoxins.

| Cyanotoxin | Regulatory Screening Level | | | | Study Screening Level is Based Upon | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Regulatory Agency | Type of Drinking Water Screening Level | Description of Screening Level | Drinking Water Screening Level (µg/L) | Endpoint | Model | Administration Method | Study Duration | POD | Health-Based Toxicity Value and Screening Level Calculation | References |
| Cylindrospermopsin | US EPA. 2015. Drinking Water Health Advisory for the Cyanobacterial Toxin Cylindrospermopsin. | 10-Day Health Advisory | Children pre-school age and younger (under 6 years old) | 0.7 | Kidney weight | Male mice | Daily oral gavage | 11 weeks | NOAEL = 30 µg/kg/day | NOAEL/300=RfD; RfD = 0.1 µg/kg-d; (0.1/0.15 L/kg/day; no RSC)=Health Advisory | Humpage, A.R., Falconer, I.R. 2003. Oral toxcity of the cyanobacterial toxin cylindrospermopsin in male swiss albino mice: Determination of no obserable adverse effect level for deriving a drinking water guideline value. Environmental Toxicology 18, 94-103. |
| | | | School-age children (6 years and older) | 3 | | | | | | NOAEL/300=RfD; RfD = 0.1 µg/kg-d; (0.1/0.03 L/kg/day; no RSC)=Health Advisory | |
| Cylindrospermopsin | WHO. 2020. Cyanobacterial toxins: cylindrospermopsins. Background document for development of WHO Guidelines for drinking-water quality and Guidelines for safe recreational water environments. WHO/HEP/ECH/WSH/2020.4. | Provisional lifetime drinking water health-based guidance value | Adult lifetime | 0.7 | Kidney weight | Male mice | Daily oral gavage | 11 weeks | NOAEL = 30 µg/kg/day | NOAEL/1,000= TDI; TDI = 0.03 µg/kg-d; (0.03 µg/kd-d*60 kg*0.8 RSC)/(2 L/d)= Guidance value | Humpage, A.R., Falconer, I.R. 2003. Oral toxcity of the cyanobacterial toxin cylindrospermopsin in male swiss albino mice: Determination of no obserable adverse effect level for deriving a drinking water guideline value. Environmental Toxicology 18, 94-103. |
| | | Provisional Guideline Value; based on short-term exposure | Adult short-term | 3.0 | | | | | | No toxicity value; (30 µg/kg-d*60 kg*1.0 RSC)/(300 UF*2 L/d)= Guideline value | |
| Cylindrospermopsin | Australia. 2018. Australian Drinking Water Guidelines 6 (NHMRC, NRMMC) | Health Alert | Due to lack of adequate data no guideline is set for cylindrospermopsin, however an initial health alert is estimated | 1 | Kidney weight | Male mice | Daily oral gavage | 11 weeks | NOAEL = 30 µg/kg/day | No toxicity value derived due to lack of data; (30 µg/kg/d*70 kg*0.9 RSC)/(2 L/day*1000 UF)= Health alert | Humpage, A.R., Falconer, I.R. 2003. Oral toxcity of the cyanobacterial toxin cylindrospermopsin in male swiss albino mice: Determination of no obserable adverse effect level for deriving a drinking water guideline value. Environmental Toxicology 18, 94-103. |
| Cylindrospermopsin | New Zealand. 2018. Drinking-water Standards for New Zealand 2005, Revised 2018; Ministry of Health. | Drinking Water Standard | Provisional maximum acceptable value | 1 | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Cylindrospermopsin | Brazil. 2009. Guidelines for Drinking Water Quality, Official LA Report's, Regulation MS N 518/2004. | Guideline for Drinking Water Quality (Recommended) | N/A | 15 | N/A | N/A | N/A | N/A | N/A | N/A | Cited in USEPA. 2015. Drinking Water Health Advisory for the Cyanobacterial Toxin Cylindrospermopsin |
| Cylindrospermopsin | Oregon Health Authority. 2019. Public Health Advisory Guidelines, Harmful Algae Blooms in Freshwater Bodies. | Drinking Water Guideline | Ages 5 years and younger | 0.7 | Kidney weight | Male mice | Daily oral gavage | 11 weeks | NOAEL = 30 µg/kg/day | NOAEL/300 = TDI; TDI = 0.1 µg/kg-d; (0.1 µg/kg-d/0.15 L/kg/day; no | Humpage, A.R., Falconer, I.R. 2003. Oral toxcity of the cyanobacterial toxin cylindrospermopsin in male swiss albino mice: Determination of no obserable adverse effect level for deriving a drinking water guideline value. Environmental Toxicology 18, 94-103. |
| | | | Adults | 3 | | | | | | NOAEL/300 = TDI; TDI = 0.1 µg/kg-d; (0.1 µg/kg-d/0.03 L/kg/day; no | |
| Cylindrospermopsin | Ohio EPA. 2020. Public Water System Harmful Algal Bloom Response Strategy | Drinking Water Threshold | Do Not Drink –children under 6 and sensitive populations | 0.7 | Kidney weight | Male mice | Daily oral gavage | 11 weeks | NOAEL = 30 µg/kg/day | NOAEL/300 = RfD; RfD = 0.1 µg/kg-d; (0.1 µg/kg-d/0.15 L/kg/day; no | Humpage, A.R., Falconer, I.R. 2003. Oral toxcity of the cyanobacterial toxin cylindrospermopsin in male swiss albino mice: Determination of no obserable adverse effect level for deriving a drinking water guideline value. Environmental Toxicology 18, 94-103. |
| | | | Do Not Drink –children 6 and older and adults | 3.0 | | | | | | NOAEL/300 = RfD; RfD = 0.1 µg/kg-d; (0.1 µg/kg-d/0.03 L/kg/day; no | |

## Table A.1 (continued). Regulatory human drinking water screening levels for cyanotoxins.

| Cyanotoxin | Regulatory Screening Level | | | | Study Screening Level is Based Upon | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Regulatory Agency | Type of Drinking Water Screening Level | Description of Screening Level | Drinking Water Screening Level (µg/L) | Endpoint | Model | Administration Method | Study Duration | POD | Health-Based Toxicity Value and Screening Level Calculation | References |
| Anatoxin-a | US EPA. 2015. Health Effects Support Document for the Cyanobacterial Toxin Anatoxin-A. | No drinking water value | Unable to derive due to lack of data | N/A | Mortality | Mice | Oral Gavage | 28 days | NOAEL = 100 µg/kg/day | N/A | USEPA 2015 discusses NOAEL, LOAEL; but data do not support deriviation of a RfD or RfC; available data do not support assessment of carcinogenic potential<br><br>Fawell, J.K., Mitchell, R.E., Hill, R.E., and Everett, D.J. (1999). The toxicity of cyanobacterial toxins in the mouse: II anatoxin-a. Human & Experimental Toxicology 18, 168–173. |
| Anatoxin-a | WHO. 2020. Cyanobacterial toxins: anatoxin-a and analogues. Background document for development of WHO Guidelines for drinking-water quality and Guidelines for safe recreational water environments. WHO/HEP/ECH/WSH/2020.1 | Provisional short-term drinking water health-based reference value | Short-term drinking water HBGV for adults | 30 | Mortality | Mice | Oral Gavage | 28 days | NOAEL = 98 µg/kg-d | No toxicity value; (98 µg/kg-d*60 kg*1.0 RSC)/(100 UF *2 L/d)=Reference value | Fawell, J.K., Mitchell, R.E., Hill, R.E., and Everett, D.J. 1999. The toxicity of cyanobacterial toxins in the mouse: II anatoxin-a. Human & Experimental Toxicology 18, 168–173. |
| Anatoxin-a | New Zealand | Drinking Water Guideline | Provisional maximum acceptable value | 6 | N/A | N/A | N/A | N/A | N/A | N/A | Drinking-water Standards for New Zealand 2005, Revised 2018 (Ministry of Health, 2018) |
| Anatoxin-a | Minnesota Department of Health. 2016. Toxicological Summary for Anatoxin-a | Short-term Non-Cancer Risk Assessment Advice | | 0.1 | Mortality | Mice | Oral Gavage | 28 days | NOAEL = 98 µg/kg-d) | NOAEL*0.14 DAF/300=RfD; RfD = 0.047 µg/kg-d; (0.047 µg/kg-d*0.8 RSC)/(0.285 L/kg-d)=Noncancer | Fawell, J.K., Mitchell, R.E., Hill, R.E., and Everett, D.J. 1999. The toxicity of cyanobacterial toxins in the mouse: II anatoxin-a. Human & Experimental Toxicology 18, 168–173. |
| Anatoxin-a | Oregon Health Authority. 2019. Public Health Advisory Guidelines, Harmful Algae Blooms in Freshwater Bodies | Drinking Water Guideline | Ages 5 years and younger | 0.7 | Mortality | Mice | Oral Gavage | 28 days | NOAEL = 100 µg/kg/day | NOAEL/1,000= TDI; TDI = 0.1 µg/kg-d; (0.1 µg/kg-d/0.15 L/kg/day; no RSC)= | Fawell, J.K., Mitchell, R.E., Hill, R.E., and Everett, D.J. 1999. The toxicity of cyanobacterial toxins in the mouse: II anatoxin-a. Human & Experimental Toxicology 18, 168–173. |
| | | | Adults | 3 | Mortality | Mice | Oral Gavage | 28 days | NOAEL = 100 µg/kg/day | NOAEL/1,000= TDI; TDI = 0.1 µg/kg-d; (0.1 µg/kg-d/0.03 L/kg/day; no RSC)= | |
| Anatoxin-a | Ohio EPA. 2020. Public Water System Harmful Algal Bloom Response Strategy | Drinking Water Threshold | Do Not Drink –children under 6 and sensitive populations | 0.3 | Increase in WBC counts | Rats | Drinking water | 7 weeks | NOAEL = 50 µg/kg/day | NOAEL/1,000=RfD; RfD = 0.05 µg/kg-d; (0.05 µg/kg-d/0.15 L/kg/day; no RSC)=Threshold value | Astrachan, N. B. and Archer, B. G. 1981. Simplified monitoring of anatoxin-a by reverse-phase high performance liquid chromatography and the sub-acute effects of anatoxin-a in rats. In: W. W. Carmichael, (Ed). The Water Environment: Algal Toxins and Health. Plenum Press, New York, NY: 437-446. |
| | | | Do Not Drink –children 6 and older and adults | 1.6 | | | | | | NOAEL/1,000=RfD; RfD = 0.05 µg/kg-d; (0.05 µg/kg-d/0.03 L/kg/day; no RSC)=Threshold value | |

## Table A.2. Regulatory dog and livestock water intake and crust/mat consumption screening levels for cyanotoxins.

| Cyanotoxin | Regulatory Screening Levels | | | | | Study Screening Level is Based Upon | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Regulatory Agency | Description of Screening Level | Species | Water Intake (µg/L) | Crust & Mat Consumption (mg/kg-dry weight) | Endpoint | Model | Administration Method | Study Duration | POD | Health-Based Toxicity Value and Screening Level Calculation | References |
| Microcystins (Includes microcystins LA, LR, RR, and YR) | Cal EPA. 2012. Toxicological summary and suggested action levels to reduce potential adverse health effects of six cyanotoxins. Office of Environmental Health Hazard Assessment (OEHHA). | Acute (<24 hrs, exposure for a single day) | Dog | 100 | 0.5 | Mortality | Sheep | Intraruminal injection | Single dose | NOAEL = 3.7 mg/kg | 3.7 mg/kg / 100 UF = RfD;<br>RfD = 37 µg/kg;<br>(37 µg/kg * 20 kg dog) / (0.085 L/kg-d *3 UF) = Water Intake;<br>(37 µg/kg * 20 kg dog)/(0.025 kg/kg *3 UF) = Crust Consumption | Jackson AR, McInnes A, Falconer IR, Runnegar MT. 1984. Clinical and pathological changes in sheep experimentally poisoned by the blue-green alga Microcystis aeruginosa. Vet Pathol; 21(1):102-13. doi: 10.1177/030098588402100117. |
| | | | Cattle (based on small breed dairy cow exposure scenario) | 50 | 5 | | | | | | 3.7 mg/kg / 100 UF = RfD;<br>RfD = 37 µgkg;<br>(37 µgkg * 450 kg cow)/ (0.23 L/kg-d *3 UF)= Water Intake;<br>(37 µgkg * 450 kg cow)/ (0.0026 kg/kg *3 UF)= Crust Consumption | |
| | | Subchronic (up to 10% of lifetime) | Dog | 2 | 0.01 | Liver lesions | Male rats | Microcystin-LR in drinking water | 28 days | LOAEL = 50 µg/kg/day | BMDL 6.4 ug/kg / 10 = RfD;<br>RfD = 0.64 ug/kg;<br>(0.64 ug/kg * 20 kg dog)/(0.085 L/kg-d *3 UF) = Water Intake;<br>(0.64 ug/kg * 20 kg dog)/(0.025 kg/kg *3 UF) = Crust Consumption | Heinze, R. 1999. Toxicity of the cyanobacterial toxin microcystin-LR to rats after 28 days intake with the drinking water. Environ. Toxicol., 14(1): 57–60. |
| | | | Cattle (based on small breed dairy cow exposure scenario) | 0.9 | 0.1 | | | | | | BMDL 6.4 ug/kg / 10 = RfD;<br>RfD = 0.64 ug/kg;<br>(0.64 µgkg * 450 kg cow)/ (0.23 L/kg-d *3 UF)= Water Intake;<br>(0.64 µgkg * 450 kg cow)/ (0.0026 kg/kg *3 UF)= Crust Consumption | |
| Cylindrospermopsin | Cal EPA. 2012. Toxicological summary and suggested action levels to reduce potential adverse health effects of six cyanotoxins. Office of Environmental Health Hazard Assessment (OEHHA). | Acute (<24 hrs, exposure for a single day) | Dog | 200 | 0.5 | Mortality | Mice | Intraruminal injection | Single dose | NOAEL = 4.0 mg/kg | 4.0 mg/kg / 100 UF = RfD;<br>RfD = 40 µg/kg;<br>(40 µg/kg * 20 kg dog)/ (0.085 L/kg-d *3 UF)= Water Intake;<br>(40 µg/kg * 20 kg dog)/ (0.025 kg/kg *3 UF)= Crust Consumption | Shaw GR, Seawright AA, Moore MR, Lam PK. 2000. Cylindrospermopsin, a cyanobacterial alkaloid: evaluation of its toxicologic activity. Ther Drug Monit; 22(1):89-92. doi: 10.1097/00007691-200002000-00019.<br><br>Shaw, G.R., A.A. Seawright, and M.R. Moore. 2001. Toxicology and human health implications of the cyanobacterial toxin cylindrospermopsin. Mycotoxins and Phycotoxins in Perspective at the Turn of the Millennium. Eds, WJ Dekoe, RA Samson, HP van Egmond, J. Gilbert, M. Sabino. IUPAC & AOAC International Brazil, p. 435–443.<br><br>Seawright AA, et al. 1999. The oral toxicity for mice of the tropical cyanobacterium Cylindrospermopsis raciborskii |
| | | | Cattle (based on small breed dairy cow exposure scenario) | 60 | 5 | | | | | | 4.0 mg/kg / 100 UF = RfD;<br>RfD = 40 K7/kg;<br>(40 µg/kg * 450 kg cow)/ (0.23 L/kg-d *3 UF)= Water Intake;<br>(40 µg/kg * 450 kg cow)/ (0.0026 kg/kg *3 UF)= Crust Consumption | |
| | | Subchronic (up to 10% of lifetime) | Dog | 10 | 0.04 | Kidney weight | Male mice | Daily oral gavage | 11 weeks | NOAEL = 30 µg/kg/day | BMDL 33 ug/kg / 10 UF = RfD;<br>RfD = 3.3 µg/kg;<br>(3.3 µg/kg * 20 kg dog)/ (0.085 L/kg-d *3 UF)= Water Intake;<br>(3.3 µg/kg * 20 kg dog)/ (0.025 kg/kg *3 UF)= Crust Consumption | Humpage, A.R., Falconer, I.R. 2003. Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male swiss albino mice: Determination of no observable adverse effect level for deriving a drinking water guideline value. Environmental Toxicology 18, 94-103. |
| | | | Cattle (based on small breed dairy cow exposure scenario) | 5 | 0.4 | | | | | | BMDL 33 ug/kg / 10 UF = RfD;<br>RfD = 3.3 µg/kg;<br>(3.3 µg/kg * 450 kg cow)/ (0.23 L/kg-d *3 UF)= Water Intake;<br>(3.3 µg/kg * 450 kg cow)/ (0.0026 kg/kg *3 UF)= Crust Consumption | |

**Table A.2 (continued). Regulatory dog and livestock water intake and crust/mat consumption guidelines for cyanotoxins.**

| Cyanotoxin | Regulatory ScreeningLevels | | | | | Study Screening Level is Based Upon | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Regulatory Agency | Description of Screening Level | Species | Water Intake (µg/L) | Crust & Mat Consumption (mg/kg-dry weight) | Endpoint | Model | Administration Method | Study Duration | POD | Health-Based Toxicity Value and Screening Level Calculation | References |
| Anatoxin-a | Cal EPA. 2012. Toxicological summary and suggested action levels to reduce potential adverse health effects of six cyanotoxins. Office of Environmental Health Hazard Assessment (OEHHA). | Acute (<24 hrs, exposure for a single day) | Dog | 100 | 0.3 | Mortality | Mice | Oral gavage | 5 days | NOAEL = 2.5 mg/kg-day | 2.5 mg/kg-day / 100 UF = RfD; RfD = 25 µg/kg-d; (25 µg/kg-d * 20 kg dog)/ (0.085 L/kg-d *3 UF) = Water Intake; (25 µg/kg-d * 20 kg dog)/ (0.025 kg/kg-d *3 UF) = Crust | 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):168-73. doi: 10.1177/096032719901800306. |
| | | | Cattle (based on small breed dairy cow exposure scenario) | 40 | 3 | | | | | | 2.5 mg/kg-day / 100 UF = RfD; RfD = 25 µg/kg-d; (25 µg/kg-d * 450 kg cow)/ (0.23 L/kg-d *3 UF) = Water Intake; (25 µg/kg-d * 450 kg cow)/ (0.0026 kg/kg *3 UF) = Crust Consumption | |
| | | Subchronic (up to 10% of lifetime) | Dog | 100 | 0.3 | | | | | | 2.5 mg/kg-day / 100 UF = RfD; RfD = 25 µg/kg-d; (25 µg/kg-d * 20 kg dog)/ (0.085 L/kg-d *3 UF) = Water Intake; (25 µg/kg-d * 20 kg dog)/ (0.025 kg/kg-d *3 UF) = Crust | |
| | | | Cattle (based on small breed dairy cow exposure scenario) | 40 | 3 | | | | | | 2.5 mg/kg-day / 100 UF = RfD; RfD = 25 µg/kg-d; (25 µg/kg-d * 450 kg cow)/ (0.23 L/kg-d *3 UF) = Water Intake; (25 µg/kg-d * 450 kg cow)/ (0.0026 kg/kg *3 UF) = Crust Consumption | |

**ATTACHMENT A**

# Literature Workbook

**ATTACHMENT B**

# Cyanotoxin Toxicity Values and Health-Based Screening Levels Workbook