Information on NJDEP cyanotoxin Reference Doses and drinking water guidance reviewed by Drinking Water Quality Institute Health Effects Subcommittee

NJDEP Reference Doses for cyanotoxins (microcystins, cylindrospermopsin, anatoxin-a, and saxitoxin):

NJDEP drinking water guidance for cyanotoxins (microcystins, cylindrospermopsin, anatoxin-a, saxitoxin):

APPENDIX 4

DERIVATION OF HEALTH ADVISORY GUIDANCE LEVELS FOR CYANOTOXINS
March 10, 2017

Derivation of Reference Doses (RfDs)

The studies summarized here appear to be the only relevant mammalian *in vivo* oral dosing studies available for each of the hazardous algal toxins addressed in this assessment. They include all of the oral exposure studies addressed by WHO and the other states referenced in appendix 3 and other relevant studies cited in the USEPA Health Advisories and Support documents for microcystins, cylindrospermopsin and anatoxin-a (USEPA, 2015a-e).

The assessment of these cyanotoxins is limited to those animal studies that administered the toxin as the specifically isolated chemical. It is also generally limited to those studies in which the toxin was administered orally, either by gavage or through drinking water. Data from studies that included injection sub-studies are included where those data are useful in informing the results of the oral administration. As explained in the Exposure Scenario section, only studies with less than sub-chronic to sub-chronic duration were considered for quantitative derivation of an RfD. Finally, these assessments are limited to studies that evaluated at least one endpoint suitable for RfD development. Studies that only presented data on sub-clinical or mechanistic endpoints are not considered here.

I. Microcystin-LR (MC-LR)

Review of toxicologic data:

Fawell et al., (1999)[2] - Mice (CD-1) were exposed to MC-LR obtained from a commercial laboratory. (This study also initially investigated rats as well as mice, but mice were found to be a more sensitive species). It was stated that MC-LR was obtained from a commercial laboratory as a "pure" substance, but no further details were provided. Fawell et al. (1994) do not indicate that the material was tested for purity. As MC-LR is a (hepta)peptide, this is somewhat less of a concern than for more structurally complex chemicals. However, this does introduce some uncertainty into the quantitative assessment of the reported results.

Single dose study: Groups of 5 male and female mice were given a single dose of MC-LR in aqueous solution by gavage (500, 1580, 5000 μg/kg) or by intraperitoneal injection (50, 158, 500 μg/kg). This study was intended as a range-finding study and no control groups were used. By

[2] The Fawell et al. (1999) paper provides a journal-based version of the report-based Fawell et al. (1994) report-based version of this research. There does not appear to be significant disagreement between these two publications, but the report-based version is more complete.

oral exposure, there was no mortality among males at the two lower doses and 60% mortality at the high dose. Among the females, there was no mortality at the low dose, and 20% and 40% at the two highest doses. By intraperitoneal (i.p.) injection there was no mortality at the lowest dose and 100% mortality for males and females at the two highest doses. The oral LD_{50}, therefore, is approximately 5,000 μg/kg.

Liver effects from the single dose study were also examined. For the oral dose, "minimal" diffuse hemorrhage was observed at the low (500 μg/kg) dose in 2/5 males, and in 1/5 at each of the two highest doses. There were also 2/5 and 5/5 observations of "moderate" centrilobular hemorrhage in 2/5 males at 1580 μg/kg and 5/5 males at 5000 μg/kg, and in 2/5 females at 5000 μg/kg. In addition, centrilobular necrosis was observed in 1/5 males and females at the highest dose. Diffuse hemorrhage was also evident at all i.p. doses.

It is clear that the liver is a target for MC-LR. The apparent LOAEL for acute liver effects is 500 μg/kg from oral exposure for minimal diffuse hemorrhage in males, with no NOAEL identified; although the lack of this effect at higher doses and with repeated dosing makes this conclusion somewhat unclear. It should be noted that diffuse hemorrhage occurred at lower doses (50 and 158 μg/kg) from i.p. injection.

Developmental study**:** Pregnant female mice (n = 14-26/dose group) were exposed by gavage from GD 6-15 to 0, 200, 600, or 2,000 μg/kg/d in aqueous solution with necropsy on GD 18. In the high dose group, 9 females died or were sacrificed *in extremis*. Body weight gain among surviving dams was not affected. No effects are reported for the dams exposed at the two lower doses. The number of implantations, live fetuses, and post-implantation loss were not clearly affected by MC-LR. There was a small (6%), but significant reduction, in fetal weight in males and females compared to controls at the highest maternal dose, but no clear effect on fetal weight at the other doses. There was no clear effect of MC-LR on fetal visceral or skeletal structure at any dose.

MC-LR does not appear to be strongly fetotoxic. The high maternal dose (2,000 μg/kg/d) appears to have a mild effect on fetal weight gain and should be considered a LOAEL for fetal effects, although this dose also resulted in maternal lethality. The NOAEL is, therefore, 600 μg/kg/d.

Less than sub-chronic duration study (14 days)**:** Male and female mice (5/sex/dose group) were exposed by gavage daily to 0, 40, 200, or 1,000 μg/kg/d (presumably in aqueous solution, as this was the method in the longer-term follow-up 13 week study) for 14 days. Histopathology was conducted on lung and liver. Organ weights were not determined. There was no dose-related mortality, and there were no effects on body weight, body weight gain, or food consumption. Macroscopic findings were not remarkable with the possible exception of red discoloration of an accentuated lobular pattern on the liver for 2/5 males at 1,000 μg/kg/d, and a red discoloration of

the lungs for 1/5 males at 40 µg/kg/d and 2/5 males at 1,000 µg/kg/d. Histopathology found hepatic centrilobular cellular rarefaction (decreased density of cell count) at 1,000 µg/kg/d.

1,000 µg/kg/day appears to be a minimal LOAEL and 200 µg/kg/d the NOAEL from this study.

Sub-chronic study (13 weeks): Males and female mice (15/sex/dose group) were exposed by daily gavage to 0, 40, 200, or 1,000 µg/kg/d in aqueous solution for 13 weeks (91 d). Animals were assessed for body weight; food consumption; hematology; blood chemistry; organ weights (adrenals, kidneys, liver and testes); organ/tissue pathology; and histopathology. For males at 1,000 µg/kg/d, there was one death on day 7 with no obvious pathology and one case of frank neurologic morbidity at day 91. There was a significant decrease in body weight gain (15%) at the 40 and 200 µg/kg/d doses, and body weight was also decreased at the 1,000 µg/kg/d dose but was not statistically significant. For females, there was a significant (29%) *increase* in body weight at the 200 µg/kg/d dose only. For both males and females, there was a significant increase in food consumption (14% and 20%) at 1,000 µg/kg/d.

In females, there was a significant increase (10-12%) in hemoglobin concentration, RBC count, and packed RBC volume at 1,000 µg/kg/d. In males, liver enzyme concentrations in blood were significantly increased at 200 µg/kg/d (ALT, AST) and 1,000 µg/kg/d (ALP, ALT, AST). There was also an overall decrease in gamma-glutamyl transferase (GGT), but this was significant only for males at 200 µg/kg/d. Total blood protein and albumin were also significantly decreased at 200 and 1,000 µg/kg/d in males. Although not significant, there was also a decrease in these two parameters at 40 µg/kg/d. No dose-related effects on organ weight were observed. Significant histopathological effects were seen only in the liver. For both males and females, there was a monotonic increase in the incidence of generalized chronic liver inflammation. The authors did not provide an analysis of statistical significance for this observation. However, a chi-squared analysis indicates that for males and females, there was a statistically significant difference only between the controls and the 1,000 µg/kg/d dose.

Although 200 µg/kg/d is a clear effect level in this study based on decreased weight gain in males, increased serum liver enzymes, and decreased total blood protein, and albumin in males, it is not entirely clear from these data that 40 µg/kg/d is a NOAEL. This conclusion is based on the significant decrease in body weight gain in males, and is also supported by changes in other parameters (total blood protein, albumin, and chronic liver inflammation) that were not statistically significant at this dose. Therefore, it appears appropriate to characterize 40 µg/kg/d as a minimal LOAEL.

Heinze (1999) - Adult male hybrid rats (WELS x BDIX) received MC-LR [Calbiochem] through drinking water at doses of 50 or 150 µg/kg/d. There were 10 animals/dose group including controls. Animals were sacrificed after 28 days of exposure. Measurements consisted of: body weights; organ weights (liver, kidneys, adrenals, thymus, and spleen); erythrocyte and leukocyte

counts; hemoglobin concentration; hematocrit; and serum enzymes. Histopathological examinations were conducted on liver and kidney.

There was no dose-related effect on body weight. Relative liver weight was significantly increased relative to controls at both doses. There were no other significant changes in organ weight. There was a significant increase in leukocytes and lymphocytes at the 150 μg/kg/d dose only. There was a significant increase in the serum levels of LDH and ALP (but not ALA or AST) at both doses, suggesting liver toxicity. Liver pathology, characterized by the author as "toxic hepatosis" was observed diffusely throughout the parenchyma at both doses. This included degenerative and necrotic hepatocytes (with and without hemorrhages).

This study yields clear evidence of liver damage at both doses and evidence of hematologic abnormalities at the high dose. Based on these observations, 50 μg/kg/d is a clear LOAEL. No NOAEL was identified from this study. It should be noted that this study was chosen by the USEPA (2015a) as the basis for its Health Advisory for MC-LR.

Li et al. (2014) – Adult male Sprague-Dawley rats were exposed by gavage to commercial MC-LR (reported as > 95% purity) mixed with methanol and diluted to administered concentrations with distilled water. The reported administered amounts were 0, 0.2, 1.0, or 5.0 μg/kg every two days for a total of 8 weeks (56 d), corresponding to effective doses of 0, 0.1, 0.5, and 2.5 μg/kg/d (n = 8/dose group). The primary analysis in this study was the potential effect of MC-LR on spatial learning as measured in the Morris water maze test administered 24 hrs after the final dosing. In addition, 24 hrs after the behavioral studies, blood was drawn and analyzed for serum liver enzymes, cholinesterase, total protein and albumin. The rats were sacrificed 24 hrs after the completion of the behavioral studies. Histopathology was conducted on the neurons of the hippocampal region of the brain, and on the liver.

Rats showed decreasing performance on most of the Morris water maze parameters as a function of dose, with significant decrements in the 2.5 μg/kg/d group and a significant decrement in the 0.5 μg/kg/d group in one parameter (platform zone frequency). Serum cholinesterase was significantly increased at 2.5 μg/kg/d. No significant treatment related histopathology was noted in either the hippocampus or the liver. However, the authors did report an increase in the density of N-20+ cells in the hippocampus as a function of dose. This effect reached statistical significance relative to the control animals only at the highest dose, however. N-20+ is an immunologic marker for the expression of the NOS2 gene that codes for nitric oxide synthetase. This gene is inducible by cytokines and the increase of N-20+ cells was interpreted by the authors as in indication of an inflammatory response in the hippocampus. The interpretation of the toxicological significance of this observation, however, is not entirely clear.

Based on the performance decrements in the Morris water maze test, 0.5 μg/kg/d appears to be a LOAEL for neurologic effects from this study, and 0.1 μg/kg/d is the NOAEL. However, as the USEPA (2015c) points out, the administered doses of MC-LR also delivered doses of methanol

that increased in proportion to the administered MC-LR dose, but given the lack of data on the volume of the gavage solution, the actual methanol dose cannot be determined. Methanol is a known neurotoxicant and the potential for a synergistic effect of methanol and MC-LR cannot be ruled out.

Li et al. (2015) – Adult female Sprague-Dawley rats (n = 7/dose group) were dosed by gavage with MC-LR (mixed with methanol and diluted with distilled water) at 0, 1.0, 5.0 or 20.0 μg/kg every two days for 8 wks. This resulted in effective doses of 0, 0.5, 2.5, or 10.0 μg/kg/d. One day following the final dosing, the rats were mated with unexposed males. Maternal body weight was recorded during gestation, and reproductive parameters were recorded. From each litter, 4 M and 4 F pups at PND 7 were evaluated in a series of behavioral tests: motor development (surface righting, negative geotaxis, cliff avoidance); and at PND 28 and 60 were evaluated for open field and Morris water maze. Histopathology was also conducted on one male and one female pup brain from each dam 24 hrs after each behavioral test.

There was no treatment related maternal toxicity and no effect on maternal body weight. The number of pregnant females appears to have declined and the number of dead fetuses appears to have increased with MC-LR dose. However, these parameters did not differ significantly from controls. Performance of the pups in the cliff avoidance test declined significantly at all doses. Pup performance in the Morris water maze was negatively affected by treatment. This was most evident with respect to the frequency-in-platform-zone parameter which showed statistically significant reduced performance at all doses. No treatment related effect was seen in brain histopathology. There was, however, an increase in markers of oxidative stress in the hippocampus that were significant at the highest dose and to a lesser extent at 2.5 μg/kg/d.

The apparent LOAEL for neurological developmental effects in this study was 0.5 μg/kg/d. There was no NOAEL. However, as with the related Lin et al. (2014) study, MC-LR exposure was associated with methanol exposure, although the actual methanol dose cannot be determined. It is, therefore, difficult to determine the specific effect of MC-LR alone. It should also be noted that the exposure of the females in this study was only pre-conception. While this does not, by itself, limit the applicability of these data for the purpose of identifying an adverse effect, it does make interpretation of the nature of the effects of MC-LR difficult. Because the pharmacokinetics of MC-LR in these animals is unknown, the fetal doses cannot be estimated.

Zhang et al. (2010) – Adult male C57bl/6 mice received commercial MC-LR in drinking water. The purity of the MC-LR was stated to be > 95%. The mice were exposed for 180 days, making this a chronic exposure study. The drinking water concentrations were 0, 1, 40 and 80 μg/L which, according to the authors, corresponded to doses of 0, 0.2, 8.0 and 16.0 μg/kg/d with n = 10 for each group. The primary purpose of this study was to investigate the effect of MC-LR exposure on the expression of matrix metalloproteinases. These are a family of enzymes that function in degrading the extracellular protein matrix. As such, they are linked to tumor progression by providing space for tumor expansion. However, because this assessment focuses

on short-term exposure, tumor progression is not considered a relevant endpoint and changes in matrix metalloproteinase expression are, therefore, considered to be a mechanistic or sub-clinical endpoint that is not appropriate for short-term RfD development, Nonetheless, this study also addresses outcome determinations relevant to RfD derivation: body weight, liver weight, and liver histopathology.

The authors report a statistically significant decrease in body weight and an increase in relative liver weight compared to the controls in the 8.0 and 16.0 μg/kg/d dose groups. However, the specific data are not presented. Histopathological examination of the livers revealed infiltration of lymphocytes and fatty degeneration in the 8.0 and 16.0 μg/kg/d dose groups.

While this study confirms the hepatotoxic potential of MC-LR, as a chronic duration study, it is not quantitatively applicable for derivation of a shorter duration RfD.

Ueno et al. (1999) - Adult female BALB/c mice were exposed to MC-LR isolated from "algal bloom materials and stated to have > 95% purity. Exposure occurred through drinking water for 3 (n = 20), 6 (n = 20), 12 (n = 20) or 18 months (n = 40) at a single concentration of 20 μg/L plus controls. The mean cumulative MC-LR intake over 18 months (548 days) was reported as 35.5 μg/animal. The mean body weight for the animals is not reported. However, the graphical presentation of body weight over the duration of exposure yields an estimated time-weighted body weight of 25.3 g (0.025 kg). Thus the dose of MC-LR can be estimated as 2.6 μg/kg/d. This study is considered a chronic duration study for all exposures except for the 3 month exposure. Analyses included body weight; organ weights; hematology; serum chemistry - liver enzymes, serum glucose, lipids, bilirubin, Ca, and inorganic P. Histopathology was performed on a large number of tissues.

There was no significant difference from controls in survival, body weight, or food or water consumption at intermediate time points or at the termination of the exposure. No significant differences were noted in hematology throughout the study. The only statistically significant treatment related effects seen in serum chemistry were a transient decrease in ALP at 12 months and an increase in total cholesterol at 18 months. A significant decrease in relative thymus weight was observed at months 3-12, but not at 18 months. A significant decrease in absolute (but not relative) heart weight was observed 18 months, but not at earlier time periods. No remarkable outcomes were observed in histopathological analysis (including histopathology of the liver)

Overall this study did not show significant adverse outcomes from MC-LR, and a dose of approximately 2.6 μg/kg/d can be considered to be a chronic NOAEL. However, given the chronic nature of this exposure, this cannot reliably be back-extrapolated to a shorter term RfD. It can, however, be useful in informing an RfD derived from a less than chronic study, especially for liver histopathology, as a NOAEL from a chronic duration study can be expected to be a lower bound estimate for a NOAEL from a less-than-chronic duration study.

Chen et al. (2011) – Adult male SPF mice (age not specified) were orally administered commercial MC-LR daily for either 3 or 6 months. The purity of the material was not provided by the authors. The route of exposure appears to be through drinking water, but this is not explicitly stated. The daily administered concentrations were 0, 1, 3.2, or 10 μg/L (n = 20/dose group). Although a range of body weights is given, the body weights over time are not specified. Thus, the dose (μg/kg/d) cannot be directly calculated from the published data. The USEPA (2015c), in its review of this study, estimates the corresponding daily doses on the basis of species/strain-specific default assumption about water intake and body weight as 0, 0.25, 0.79, and 2.5 μg/kg/d. The 6-month duration exposure is considered to be a chronic duration study. Body weight and testis weight were measured. Sperm count and sperm morphology were assessed, and testis histopathology was evaluated. Serum reproductive hormones were also measured.

No treatment-related effects were observed on body weight or testis weight. At 3 months, a significant decrease in sperm motility was observed at 0.79 and 2.5 μg/kg/d. At 6 months, there was a decrease in sperm count and sperm motility as well as an increase in the frequency of abnormal sperm for 0.79 and 2.5 μg/kg/d. Although serum testosterone appears to have markedly declined for 2.5 μg/kg/d at three months, it apparently did not reach a level of statistical significance. At 6 months, serum testosterone was statistically significantly decreased at 0.79 and 2.5 μg/kg/d. Also, LH was significantly increased at the same doses, while serum FSH was significantly increased at 2.5 μg/kg/d. Histopathology revealed a slight effect on the arrangement of spermatogenic epithelium in the seminiferous tubules at 2.5 μg/kg/d at 3 months. At 6 months, there was slight testicular atrophy at 0.79 μg/kg/d with increasing severity and various morphological abnormalities at 2.5 μg/kg/d.

This study suggests the potential for male reproductive toxicity from chronic MC-LR exposure with an apparent NOAEL of 0.25 μg/kg/d. However, a number of factors render this study problematic for RfD development. As noted above, the dose (μg/kg/d) could not be determined directly from the published data. The age of the mice was not provided and sperm quality can vary as a function of age. There are several potentially significant methodological issues with sperm and tissue analysis, as pointed out by the USEPA (2015c).

Lin et al. (2016) - This was a cross sectional epidemiology study in a population in Southwest China exposed to both MC-LR and aflatoxin. Renal function indicators (blood urea nitrogen, BUN; serum creatine, SCr; estimated glomerular filtration rate, eGFR) were evaluated as a function of estimated MC-LR (and aflatoxin) intake from water and food. The population in this study was apparently exposed long-term (possibly over a lifetime) to microcystin-LR that appears to have been chronically (or at least repeatedly) present in its environment. The mean, median, 75th and 95th percentiles of estimated daily MC-LR intake were 4.05, 3.23, 4.66, and 9.55 ng/kg/d, respectively.

For both the full study population (5,493 people) and the subset of those with abnormal renal function (129-383 people depending on the specific renal function parameter), there was a significant association of renal function indicators with estimated MC-LR (but not aflatoxin), with an apparent dose-response relationship across quartiles (significant for trend) of MCL-LR exposure. This association was seen for each renal function indicator in adjusted models. The odds ratio (OR) for having abnormal renal function indicators was significantly > 1.0 for the third and fourth quartiles of estimated MC-LR intake. The OR for having abnormal renal function indicators relative to the median estimated MC-LR intake in the fully adjusted model was significantly > 1.0 for all three renal function indicators. Although there is documented exposure to aflatoxin in this population, this did not appear to confound the observed associations with MC-LR.

Although this study does not readily lend itself to the calculation of a NOAEL, based on the above summary, the lowest quartile of exposure (i.e., 0.003 μg/kg/d) appears to be the most reasonable estimate of the estimated exposure that is not clearly associated with adverse effects. Nonetheless, the reliability of the exposure estimates in this study is not clear and there was no independent estimate of intake that could be used to ground-truth these estimates. Thus, the quantitative determination of dose-response from this study can be viewed as only suggestive. Importantly, this study suggests that MC-LR is associated with adverse renal function in humans.

RfD Derivation:

Selection of critical study for derivation of an RfD – General considerations

The two Li et al. studies (2014, 2015) on rats both suggest the potential for MC-LR to cause both adult and developmental neurotoxicity with sub-chronic exposure. The apparent LOAEL for both endpoints is 0.5 μg/kg/d. However, interpretation of the results of both studies is complicated by co-exposure to methanol and the possibility that the co-exposure could result in a synergistic response.

The Chen et al. (2011) study suggests the potential for male reproductive effects with an apparent NOAEL 0.25 μg/kg/d. However, as discussed above, there are numerous reporting and methodological issues with this study.

The Lin et al. (2016) study has the advantage of investigating a human population. However, the accuracy of the microcystin-LR exposure estimates in that study is unknown. Furthermore, the statistical analysis does not readily lend itself to the estimate of a NOAEL. In addition, although not entirely clear from the published paper, it appears that this population was chronically exposed to microcystin-LR. Thus, the estimated dose-response relationship from this study may not be appropriate for the purposes of deriving a short-term RfD. Both the Fawell et al. (1994/1994) and Heinze (1999) studies provide appropriate toxicological data for the derivation of an RfD. These studies yield a LOAEL at very similar doses, with no NOAEL identified.

In the Fawell et al. (1994/1999) study, the 40 μg/kg/d dose is identified as a minimal LOAEL based on the significant decrease in body weight gain in males, and is also supported by changes in other parameters (total blood protein, albumin, chronic liver inflammation) that were not statistically significant at this dose, but are predictive of significant effects at higher doses. This study also provides information on effects from developmental exposures. In the Heinze (1999) study, the 50 μg/kg/d dose is a clear LOAEL that reflects liver toxicity based on increased liver weight and elevated serum liver enzymes. Liver toxicity was also observed in the Fawell et al. (1994/1999) study, but only appears to have reached statistical significance at 1,000 μg/kg/d. Although the LOAEL from the Fawell et al. (1994/1999) study (40 μg/kg/d) is slightly lower than the LOAEL from the Heinze (1999) study (50 μg/kg/d), the LOAEL from Fawell et al. (1994) is judged to be a minimal LOAEL, whereas the LOAEL from Heinze (1999) is a LOAEL for more significant adverse effects. In addition, the length of exposure in the Heinze (1999) study (28 days) was less than that in the Fawell et al. (1994/1999) study (91 days). However, the USEPA (2015a) cites a study (Guzman and Solter, 1999) in which rats were intraperitoneally infused with MC-LR. In that study, the route of exposure resulted in direct exposure of the liver. Adverse liver effects were observed in that study, and the NOAEL and LOAEL doses were separated by a factor of two. The USEPA thus argues that a full factor of 10 is not necessary to estimate the NOAEL from the observed LOAEL for adverse effects in the Heinze (1999) study, and the alternative UF of 3 is appropriate. Given these considerations it appears more appropriate to identify the minimal, but lower LOAEL of 40 μg/kg/d for small, but significant decreased body weight in male mice in Fawell et al. (1994/1999) as the point of departure for RfD derivation, noting that applying a UF of 3 to estimate the NOAEL from the minimal LOAEL in Fawell et al. (1994/1999) also adequately addresses the NOAEL for liver effects in the Heinze (1999) study based on the argument presented by the USEPA (2015a).

Uncertainty factor analysis - A total UF of 3,000 was applied to the LOAEL based on the following individual UFs:

UF – study duration = 1
Although this was a sub-chronic duration study, it appears appropriate to the relevant exposure scenarios.

UF – LOAEL-NOAEL = 3
The moderate decrease in male body weight gain at the 40 μg/kg/d dose is identified as a minimal LOAEL.

UF – animal-human = 10
Standard assumption - this includes factors of 3 each for interspecies toxicokinetic and toxicodynamic variability.

UF – sensitive human populations = 10
Standard assumption – includes children as a sensitive group.

UF – database = 10

The only studies that address neurotoxicity/developmental neurotoxicity are the two studies of Li et al. (2014, 2015). Both of these studies yield a LOAEL of 0.5 μg/kg/day. The interpretation of both of these studies is potentially confounded by co-exposure to methanol, a known neurotoxicant. However, the extent of confounding by methanol in either Li et al. study is unknown, and the neurotoxicity and developmental neurotoxicity effects in these studies could, in fact, be independent of the methanol exposure. If it is assumed that the application of a UF of 3 to account for the use of a minimal LOAEL in the absence of a NOAEL from the Fawell et al. (1994/1999) study appropriately estimates a NOAEL from that study (i.e., 13 μg/kg/d), then application of an additional UF of 3 for database uncertainty would yield a value of 4 μg/kg/day (leaving aside the other UFs that are independent of the treatment of the Li et al. studies). This would still be an order of magnitude larger than the LOAEL from the Li et al. (2014, 2015) studies. Alternatively, application of a full uncertainty factor of 10 (rather than 3) to the estimated NOAEL (13 μg/kg/d) from Fawell et al. (1994/1999) to account for database uncertainty regarding the potential for neurotoxicity/developmental toxicity would result in a value of 1.3 μg/kg/d. This value is still approximately twice the LOAEL from the Li et al. studies. The application of a full UF of 10 for database uncertainty also appears to address all other database issues.

UF-total = 3,000

RfD calculation

RfD = LOAEL ÷ UF-total
= 40 μg/kg/d ÷ 3,000
= 0.013 rounded to **0.01 μg/kg/d**

Comparison to USEPA RfD and WHO Tolerable Daily Intake (TDI) - The RfD of 0.01 μg/kg/d is smaller than the Tolerable Daily Intake (TDI) (0.04 μg/kd/d) developed by WHO (2003b) as well as the RfD value developed by the USEPA (2015a) for MC-LR of 0.05 μg/kg/d. The difference is almost entirely due to the consideration of the database uncertainty regarding the potential for neurotoxicity/developmental neurotoxicity introduced by the Li et al (2014, 2015) studies. This uncertainty was not addressed directly by either WHO or USEPA.

II. Cylindrospermopsin (CYN)

Review of toxicological data:

Humpage and Falconer (2003) - Male mice (Swiss albino) were dosed with cylindrospermopsin derived from *C. raciborskii* cells (strain AWT 205) in overlapping studies, the first with

exposure by drinking water for 10 weeks with a CYN dose range of 216-657 μg/kg/d, and the second by gavage for 11 weeks with a dose range of 30-240 μg/kg/d. The drinking water study was conducted using a crude cell extract, while the gavage study utilized a purified extract, assayed as 47% CYN, 53% phenylalanine. The n for controls was 12 in the drinking water study was 12 and 10 for the gavage study. The n was 10 for all dose groups except the highest doses, which had an n of 5-6. The study protocols appear valid although the body weights of the controls at sacrifice in the two studies differed despite being in the same range at the start of dosing. There was a 23% decrease in body weight of gavage controls compared to the drinking water controls. The authors speculate that this was due to discomfort resulting from the gavage treatment *per se*.

In the drinking water study, body weight was significantly decreased at the two highest doses, and liver and kidney weight were significantly increased at all doses. Spleen weight was not significantly affected. At the two lowest doses, 30 and 60 μg/kg/d, in the gavage study, body weight was significantly increased compared to controls, but it was significantly decreased at the two highest doses in the drinking water study. Relative liver weight increased monotonically with an apparent LOAEL of 216 μg/kg/d in the drinking water study, and a NOAEL and LOAEL of 120 μg/kg/d and 240 μg/kg/d, respectively, in the gavage study. In the drinking water study, with kidney weight increased monotonically and significantly across all dose groups compared to controls. In the gavage study, kidney weight increased monotonically, with a LOAEL of 60 μg/kg/d and a NOAEL of 30 μg/kg/d (the lowest dose). There were no other significant changes in organ weight. Urine protein/creatinine ratio decreased monotonically with a LOAEL of 120 μg/kg/d in the gavage study. There was minimal liver histopathology (unspecified) at 120 and 240 μg/kg/d, but no changes in serum liver enzymes in the gavage study. Serum bilirubin was significantly increased and serum total bile acids were significantly decreased at 216 and 432 μg/kg/d in the drinking water study, but were not significantly altered in the gavage study.

The authors hypothesize that CYN inhibits protein synthesis. Decreased urinary protein (presumably small proteins, not reflective of glomerular or tubular damage) is consistent with this hypothesis, although the decreased protein/creatinine ratio in conjunction with decreased creatinine concentration at the high dose in the gavage study could reflect increased creatinine due to protein catabolism as well as, or instead of, decreased overall protein synthesis at the high dose.

The study NOAEL is 30 μg/kg/d based on increased relative kidney weight. While increased relative kidney weight is considered an adverse effect, the mode of action leading to the effect in this study is unclear. The authors hypothesize that increased kidney weight could reflect an increase in cellular volume or cellularity in response to inhibition of protein and general metabolic synthesis. While this explanation appears to be speculative, the linkage of this effect to more frank adverse effects at higher doses including decreased body weight argues for the

validity of this NOAEL. However, there would be more confidence in this value if there had been a lower dose that also showed no adverse effect.

Chernoff et al. (2011) – Pregnant CD-1 mice were exposed to commercial CYN (>98% pure) by intraperitoneal injection daily for 5 days at doses of 50 μg/kg/d during GD 8-12 or GD 13-17. Animals were sacrificed (n = 2-5) 24 hrs after the final injection, or on post-treatment days 7 and 14. Measurements included maternal weight, serum chemistry, and histopathology of liver and kidney.

Decreased weight gain in the GD 8-12 group occurred starting with the first dose, followed by vaginal bleeding, reduced activity, blood accumulation in the tail, and hemorrhaging around the eyes. There was mortality and morbidity during dosing with some mortality through GD 18. Gestational length was not affected. These effects were milder in the GD 13-17 group. Treated animals in this group gave birth earlier in the day compared to controls. Serum liver enzymes (AAT, AST, and ALT) were significantly elevated in the GD 8-12 group and in the GD 13-17 group. LDH and SDH were elevated in the GD 13-17 group. Serum albumin was significantly reduced in both groups. BUN and creatinine were significantly increased in the GD 8-12 group, and serum glucose was significantly decreased in both groups. Blood chemistry parameters returned to normal values 7 days post-dosing in both groups. Relative liver weight was not affected in the GD 8-12 group, but was elevated in the GD 13-17 group and did not recover. Minimal-moderate centrilobular hepatocyte necrosis and apoptosis was elevated in both treatment groups compared to controls. Elevated minimal-moderate chronic interstitial nephritis was seen in the GD 8-12 group.

The injection route of exposure and the single dose nature of this study preclude its use in RfD derivation. However, this study provides evidence of the potential of CYN to result in adverse metabolic, liver and kidney effects.

Chernoff et al. (2014) - Pregnant CD-1 mice were exposed to CYN by i.p. injection during either gestation day (GD) 8-12, or GD 13-17. Animals were dosed on five successive days with 50 μg/kg/d of CYN. Groups of dams were sacrificed after each day of dosing and at various times up to 13 weeks post-dosing. Maternal body weight, visual signs of toxicity, serum chemistry, and liver and kidney histopathology were analyzed. The number of animals examined (generally 4-21) varied by endpoint and number of doses.

Maternal weight decreased significantly during the dosing period in both gestational period groups. Vaginal hemorrhaging and visual signs of morbidity were also observed in both groups, although the late gestational period group showed effects after the first dose compared to the third dose in the early gestational group. ALT, SDH, and total bile acids were significantly increased in both groups both during, and to varying extents, post-dosing, indicating liver toxicity. Hemoglobin and hematocrit were reduced in both groups with a greater sensitivity in the later gestational group. Significant increases in liver weight were seen in the late gestational

group. Liver histopathology including hepatocellular necrosis; hepatocellular cytoplasmic alterations; and chronic centrilobular inflammation was observed in both groups. In the kidney, acute tubular necrosis was also observed in both groups. In addition, a significant decreased platelet count was observed during the last two days of dosing.

The injection route of exposure and the single dose nature of this study preclude its use in RfD derivation. However, this study provides further evidence of the potential of CYN to result in metabolic, liver, kidney and hematologic adverse effects.

de Almeida et al. (2013) – Pregnant Wistar rats were exposed to CYN by gavage at 0, 0.03, 0.3 and 3.0 μg/kg/d for GD 1-20 (n = 10/dose group). Body weight was measured during and at the end of treatment. Organ weights were measured for ovaries, uterus, kidney, pancreas, adrenal gland, heart and spleen. Histopathology was conducted on liver and kidney. Reproductive parameters were recorded. Half of the fetuses from each litter were examined for visceral malformation, and the other half were examined for skeletal malformation.

No significant differences from controls were observed for body weight or histopathology. No differences were observed in organ weights, or in the incidence of visceral or skeletal malformations.

This study yields a free-standing NOAEL of 3.0 μg/kg/d for maternal, reproductive and teratological effects from gestational exposure.

Sukenik et al. (2006) – Four-week old ICR mice (M and F) were exposed to a cell-free, but unpurified solution of CYN in water. The exposure protocol in this study is somewhat unusual. In order to minimize the number of animals, the authors gradually increased the dose to all of the animals over the course of the study. Blood was drawn from the tails every four days for determination of hematocrit and cholesterol and half of the animals were sacrificed at 20 weeks and the remainder at 42 weeks. The authors state that the dose increased from 10 μg/kg/d at the start of the study to > 50 μg/kg/d in the last 22 weeks of exposure. Although not explicitly stated in the paper, the dose at the 20-week sacrifice was approximately 30 μg/kg/d based on the graphic presentation in the paper. This study is, therefore, a chronic duration study. The authors state that analysis of the drinking water revealed only CYN and the related compound 7-epi/CYN. However, the purity of the CYN is not known. Blood samples were obtained every four weeks. There were initially 20 males and 20 females in the control and exposure groups. Liver, spleen, kidney and testes were weighed and examined by histopathology. Cholesterol was determined in RBC membranes, plasma, and liver homogenate.

No effect on body weight gain was observed. Relative liver weight was significantly increased at 42 weeks (but not at 20 weeks) in M and F. Relative kidney weight was significantly increased in M and F at both 20 and 40 weeks, and relative testes weight was significantly increased at 42 weeks. Relative spleen weight was not affected. Hematocrit was significantly increased compared to controls in M at all time periods of measurement except for the final (36

week) period when it was significantly decreased compared to controls. Female hematocrit was also significantly increased for all time periods except for the final measurement period (36 weeks). This was accompanied by deformed RBCs. RBC membrane cholesterol was significantly increased in M and F at 42 weeks. Plasma cholesterol was slightly (but significantly) increased in F at 42 weeks. In liver homogenate, however, cholesterol was significantly decreased in M at 20 weeks and in M and F at 42 weeks.

This study suggests that chronic exposure to moderate levels of CYN can result in adverse effects in liver, kidney and testes weight, hematologic parameters and cholesterol levels. However, the chronic nature of this study renders it not appropriate for derivation of a shorter term RfD. Additionally, the inability to link effects to specific CYN doses and the potential contribution of the 7-epi/CYN compound precludes the use of this study for quantitative assessment for CYN.

Rogers et al. (2007) - Pregnant CD-1 mice were injected (i.p.) with 8-128 μg/kg/d CYN, stated to be free from organic impurities and with >98% purity, during GD 8-12. Term fetuses were examined for viability and structural abnormalities. Significant lethality in the dams was observed for doses > 32 μg/kg/d, but there were no adverse effects on litter size, fetal weight, or incidence of anomalies. However, as the number of surviving dams at doses > 32 μg/kg/d was small, the number of fetuses available for evaluation at the larger doses was also small and conclusions about lack of fetal effects at doses > 32 μg/kg/d are weak. Subsequently, dams were injected with 50 μg/kg/d during GD 8-12 or 13-17. Maternal toxicity, including lethality and hemorrhaging, was noted in dams exposed during both stages of gestation although the incidence and severity was less for exposure during the later period. In the dams exposed during the later period, birth occurred earlier in the day compared to controls. A reduction in litter size compared to controls was noted for exposure during both gestational periods. Pups of dams exposed during GD 13-17 had significantly reduced body weight. There was decreased fetal survival among the pups in the GD 13-17 dosing group and indication of gastrointestinal hemorrhage. Following cross-fostering to control dams, pups of dams exposed during GD 13-17 (but not GD 8-12) had decreased viability and weight gain.

The injection route of exposure is not appropriate for deriving an oral RfD. In addition, the design of this study is not amenable to deriving a useful LOAEL or NOAEL. However, this study does provide qualitative evidence of reproductive/developmental effects of CYN.

Reference Dose (RfD) derivation:

Selection of critical study – Only two repeat dose studies with specific estimates of daily dose were identified, Humpage and Falconer (2003) and de Almeida et al. (2013). The de Almeida et al. (2013) study, however, yields only a freestanding NOAEL that is an order of magnitude lower than the NOAEL from Humpage and Falconer (2003). The Humpage and Falconer (2003) study with a critical effect of increased relative kidney weight is, therefore, the more appropriate study

for the derivation of a short term RfD. The Humpage and Falconer (2003) study is also the study selected by the USEPA for its Drinking Water Health Advisory for cylindrospermopsin (USEPA, 2015b). This study yields a NOAEL of 30 µg/kg/d based on increased relative kidney weight.

Uncertainty factor (UF) analysis - A **total UF of 1,000** was applied to the NOAEL based on the following individual UFs:

UF – study duration = 1
Although this was a less-than sub-chronic duration (11 week) study, it is consistent with the range of study durations applicable to the derivation of a short-term RfD (see Exposure Scenario section).

UF – LOAEL-NOAEL = 1
The study yields a reasonable estimate of the NOAEL.

UF – animal-human = 10
Standard assumption - this includes factors of 3 each for interspecies toxicokinetic and toxicodynamic variability.

UF – sensitive human populations = 10
Standard assumption – includes children as a sensitive group.

UF – database = 10
It is noted that the USEPA (2015b) applied an uncertainty factor of 3 for database insufficiency for CYN citing the same study. The study of Rogers et al. (2007) provides evidence that CYN can produce reproductive/developmental effects. However, the nature of this study does not permit the derivation of a meaningful LOAEL or NOAEL. Furthermore, there are no data that permit an assessment of potential neurological or immunologic effects. Thus, although there is evidence indicating that CYN is capable of causing reproductive/developmental effects, there is no basis for deriving a reproductive/developmental-specific RfD, and there is no basis for determining the NOAEL based on increased relative kidney weight is protective against reproductive/developmental effects. Based on this consideration as well as the lack of data on possible neurological and immunological effects, a full UF of 10 for database insufficiency appears justified.

UF - total = 1,000

Calculation of RfD

RfD = NOAEL ÷ UF-total
= 30 µg/kg/d ÷ 1,000
= **0.03 µg/kg/d**

Comparison to USEPA RfD - USEPA (2015d) derived an RfD of 0.1 μg/kg/d for CYN. The USEPA RfD uses the same NOAEL of 30 μg/kg from Humpage and Falconer (2003), but differs in applying a total UF of 300 rather than the value of 1,000 derived here. The basis for this difference is described above.

III. Anatoxin-a

Review of toxicological data:

Astrachan and Archer (1981) - Anatoxin-a was isolated from NRC-44-1 strain of *A. flos-aquae*. Anatoxin-a is known from previous work (Carmichael et al., 1975) to be a neurotoxin acting as a nicotinic agonist acting by stimulation followed by a depolarizing blockade to produce death with ataxia and convulsion. Based on Carmichael et al. (1975), the single dose injection (intraperitoneal) LD_{50} is approximately 250 μg/kg in rats and mice.

Female adult Sprague-Dawley rats were exposed to anatoxin-a through either drinking water (0, 51 or 510 μg/kg/d, 20/dose group) for up to 7 weeks, or by i.p. injection (0 or 89 μg/kg/d for 21 days, 18/dose group). The oral doses are estimated to be 0.8% and 8% of the oral LD_{50} respectively, and the intraperitoneal dose was estimated by the authors to be 25% of the i.p. LD_{50}. Based on the body weight provided in study, however, this dose appears to be 36% of the i.p. LD_{50}. Animals exposed by both routes were assessed for body weight, food consumption, behavior; gross lesions; liver, spleen, kidney weight, and histopathology of these organs; RBC and WBC count; and serum liver enzymes (cholinesterase, AP, SGTP, GGTP).

No significant differences were observed between the control and dosed groups in any of the parameters assessed. Although there was a transient increase in white blood cell count in the high dose animals at 5 weeks, this parameter was not different from the control or low dose value at 7 weeks, and the significance, if any, of this observation is unclear. Thus, 510 μg/kg/d is identified as a free-standing NOAEL from this study. The USEPA (2015e) identifies the high dose in this study as a LOAEL (rather than a NOAEL) on the basis of the transient white blood cell count. Although most of the data are presented only as summary narrative statements, the study design and results appear to be valid. However, there would be more confidence in the NOAEL if this study had identified a LOAEL.

Astrachan et al. (1980) - Teratology studies - Astrachan et al. (1980) isolated anatoxin-a from a laboratory culture of NRC-44-1 and reported that it was "essentially pure" by TLC and HPLC. Pregnant golden hamsters were dosed by i.p. injection on either GD 8-11, or 12-14. The only single daily dose was 200 μg/kg on GD 8-11. Other doses consisted of multiple daily injections (3 x 125 μg/kg = 375/μg/kg/d; and 3 x 200 μg/kg = 600 μg/kg/d) with an identical schedule for GD 8-11 and GD 12-14. Dams were sacrificed on GD 15 and the fetuses examined.

The authors do not report statistical significance for fetal resorption. However, for all dose groups and each gestation period, the percent resorption was greater than for controls – including greater than four-fold the control rate (GD 8-11, 375 μg/kg/d). Fetal weight was significantly decreased compared to controls for all dose groups for dosing on GD 8-11, and for the 375 μg/kg/d group on GD 12-14. The authors refer to "stunting" of the fetuses. This refers to reduced body weight. In addition, in one litter with maternal dosing at 375 μg/kg/d, all 10 of the fetuses had hydrocephaly. No other soft tissue or skeletal malformations were noted.

The injection route of exposure as well as the absence of a NOAEL preclude the direct use of this study in the derivation of an RfD. In addition, precise interpretation of these findings is hampered by incomplete and inexact reporting. Nonetheless, this study provides evidence that anatoxin-a exposure during gestation can have developmental effects including, at a minimum, decreased fetal weight.

Fawell and James (1994)/Fawell et al. (1999)[3]:
In each of the studies in this publication, Fawell and James (1994) used commercial anatoxin-a hydrochloride. The doses given below reflect adjustment to the dose as anatoxin-a (i.e., the parent molecule without the hydrochloride salt).

Single dose studies - Male CD-1 mice received anatoxin-a hydrochloride by a single intravenous injection (6 per dose group) at doses of 0, 8.2, 24.5 and 81.7 μg/kg. Mice were observed through 4 hrs post-injection and evaluated according to the Irwin protocol (a checklist of *in vivo* clinical observations). No observed effect occurred for the 8.2 or 24.5 μg/kg doses. Two animals in the 25 μg /kg dose group died and those that survived showed increased salivation, respiration and hyperactivity. No effects were observed in the low dose group (8 μg/kg anatoxin-a/kg). At 81.7 μg/kg, all animals died within 1 min of dosing with neurological symptoms consistent with a cholinergic effect. It should be noted that only a factor of approximately 3 separates the no-effect dose (relative to these parameters) from the lethal dose.

Male CD-1 mice received intravenous anatoxin-a as a single injection of 0, 24.5, 40.8, or 49 μg/kg (6/dose group). Animals were tested on the rotarod 15 min post injection. One animal died at 24.5 μg/kg, and two animals died at 40.8 μg/kg. Animals at 49 μg/kg experienced convulsions, hypersalivation, micturition, an elevated tail, and hyperactivity, and all of them died within one minute of dosing. The "majority" of animals at 24.5 μg/kg and all animals at 40.8 μg/kg had increased respiration with a duration of approximately 1 minute. Recovery of all surviving animals occurred within a few minutes. All surviving animals remained on the rotarod, indicating retention of significant neuromuscular function/coordination. Contrary to the

[3] Fawell et al. (1999) is a reiteratioin of a portion of the studies in Fawell and James (1994). The former is a journal article and the latter is a study report.

earlier intravenous injection study in this paper, 24.5 µg/kg is a LOAEL not a NOAEL here due to the apparent dose-related death at this dose.

Repeated dose studies - Two male and two female mice were dosed five times by oral gavage at each of the dose levels below. Although not stated explicitly, it appears that surviving animals were sacrificed 24 hrs after the last dose. For animals receiving 1,225 or (apparently) 2,450 µg/kg/d, dosing was on successive days. For the 6,125 and 12,250 µg/kg/d doses, dosing was every other day. All animals receiving 12,250 µg/kg/d and one female receiving 6,125 µg/kg/d died within 3 min. of dosing. At 6,125 µg/kg/d, males were hyperactive after the third dose. Body weight and food consumption were unaffected and no unusual results were observed on necropsy. No toxicity was observed at 1,225 or 2,450 µg/kg/d.

In a longer duration component of this study, mice (10 males and 10 females per dose group) received 0, 98, 490, or 2,450 µg/kg/d by gavage for 4 wks. Animals were bled without sacrifice during the final week of dosing and assessed for hematology and blood chemistry, and weight and histopathology of multiple organs was assessed at sacrifice.

One animal receiving 98 µg/kg/d was reported to have died with evidence of infection. One male receiving 490 µg/kg/d and one female receiving 2,450 µg/kg/d died within 2.5 hrs of dosing with no obvious pathology. Although there is no clear evidence linking these two deaths to the dosing, the timing relative to receiving the doses in both cases is suspicious and such a connection cannot be dismissed. There was no dose-related effect on body weight, body weight gain, or ophthalmoscope examination.

Small, but statistically significant increases in mean cell hemoglobin (Hb) (males) or mean cell Hb concentration (females) were observed. However, the increase relative to controls was small (5-6%) and the significance of this observation is unclear. A relatively large (maximum = 30%), but not statistically significant, increase in AST was observed in males at the two highest doses. However, none of the other serum liver enzymes concentrations (ALP, ALT) were remarkable, and there was no abnormal liver histopathology. There was also a relatively small (2%), but statistically significant, increase in serum Na in females. There was no significant effect on body weight or organ weight and no remarkable histopathology.

In a developmental toxicity component of this study, time-mated female CD-1 were gavage dosed once per day at 2,450 µg/kg/d (n = 10), or with sterile water (controls) during GD 6-15 (n = 12). Dams were observed for clinical signs. On GD 18, animals were sacrificed and maternal necropsy was performed. Fetal implants, live and dead fetuses, fetal weight, sex and external abnormalities were recorded. Results were only provided in narrative form with few quantitative specifics.

No maternal toxicity was observed, including no effect on body weight or weight gain. No abnormalities were noted on necropsy. Implantations, live fetuses, post-implantation losses and

sex ratio were unaffected. Mean fetal weight was "marginally" lower in the exposed group. The authors attribute this to small differences in litter size. No differences in physical abnormalities were observed between control and treated fetuses. This dose can be considered a NOAEL for developmental effects.

Although there were no clear adverse toxicological results at any of the doses, the two unexplained deaths at 490 and 2,450 μg/kg/d dictate that the clearest NOAEL is 98 μg/kg/d. A NOAEL of 98 μg/kg/d is reasonably consistent with a single dose LOAEL of 24.5 μg/kg from the i.v. injection study given the likely difference in toxicokinetics between the i.v. and gavage routes of exposure. However, it should be noted that in both studies, the LOAEL is based on lethality. This NOAEL is smaller than the freestanding NOAEL of 510 μg/kg/d in rats via drinking water from the study of Astrachan and Archer (1981).

Yavasoglu et al. (2008) - Male mice (strain not specified) were exposed by intraperitoneal injection for 7 consecutive days to 0, 50, 100, or 150 μg/kg/d of anatoxin-a. Animals were evaluated for sperm count and light microscope histopathology of the testes. There were no significant effects on body weight. However, epididymis weight decreased monotonically with significant decreases compared to controls at the two higher doses. Sperm count also decreased monotonically with all doses resulting in a significant decrease relative to controls. Histopathological changes in the testes were reported. Although counts for individual effects were not reported, the effects (including degeneration of seminiferous tubules, dissociation of spermatogenic cells with resulting sloughing of germ cells into the lumen, vacuolization of Sertoli cells, and loss of germ cells) were stated to be greater at 150 μg/kg/d than at 100 or 50 μg/kg/d. The epithelial thickness of the seminiferous tubules decreased monotonically with dose and was significantly less than controls at all doses. The injection nature of the dosing in this study precludes the quantitative use of these data in RfD derivation. However, all of the doses produced adverse effects and these results raise concerns for potential effects on male fertility with oral exposure.

RfD Derivation:

Selection of critical study – The following table summarizes the NOAELs and LOAELs for lethality from the available studies.

Study	**NOAEL** (μg/kg/d)	**LOAEL (lethality)** (μg/kg/d)	**Route of Exposure**
Astrachan and Archer (1981)	510 (freestanding)		Drinking water
Fawell and James (1994)/ Fawell et al. 1999)	98	490	gavage
Fawell and James (1994)/ Fawell et al. (1999)		24.5	intravenous

Anatoxin-a appears to cause lethality through a rapid onset neurologic toxicity that produces hyperactivity and convulsions. Following intravenous injection (Fawell and James, 1994), lethality occurred within 1 min. Following gavage exposure, lethality occurred in 3 min. (Fawell and James, 1994). Thus, the much larger NOAEL from Astrachan and Archer (1981) is likely due to the slower absorption and distribution following drinking water exposure (which is directly analogous to incidental ingestion while swimming). This argues that LOAELs for lethality based on intravenous or gavage routes of exposure are likely to be conservative estimates of the LOAELs for lethality by the oral (drinking water) route of exposure. However, there is insufficient information on which to estimate the ratio between the oral NOAEL and the (unobserved) LOAEL for lethality. Furthermore, although the recreational (i.e., swimming) exposure scenario utilized in this assessment (see below) is based on empirical data, it cannot account for unusual (but possible) short-term high volume ingestions while swimming. Therefore, given the potential for lethality, conservative (i.e., protective) assessment of the relationship between the oral NOAEL and the LOAELs for lethality from the other exposure routes, particularly gavage, is appropriate.

The free standing NOAEL of 2,450 μg/kg/d (maternal exposure) from the developmental toxicity study of Fawell and James (1994)/Fawell et al. (1999) is the largest of the NOAELS. However, that NOAEL is based on a study with limited data reported, including inadequate reporting of the maternal pathology analyses that were carried out. The next largest NOAEL of 510 μg/kg/d from the 7 week drinking water study in rats of Astrachan and Archer (1981) reflects more complete analyses and reporting. However, this dose is close to the LOAEL of 490 μg/kg/d from the 4 week repeated dosing gavage study in mice of Fawell and James (1994)/Fawell et al. (1999). In addition, although the 510 μg/kg/d dose in the Astrachan and Archer (1981) study is assessed here as a NOAEL, it could be argued (per USEPA, 2015e) that this is a minimal LOAEL (for increased white blood cells) rather than a NOAEL. The endpoint yielding the LOAEL from the Fawell and James (1994)/Fawell et al. (1999) study is lethality that is, presumably, dose-related. The possibility that the observed lethality is dose-related is supported by the LOAEL from the i.v. single dosing portion of this study (i.e., 24.5 μg/kg) that is also based on lethality. Thus, the free standing NOAEL (drinking water) from Astrachan and Archer (1981) differs from the LOAEL (gavage) of 490 μg/kg/d for lethality from Fawell and James (1994)/Fawell et al. (1999) by only 16% and does not provide sufficient protection against lethality. The NOAEL of 98 μg/kg/d from Fawell and James (1994)/Fawell et al. (1999) is a factor of five below the LOAEL (based on lethality) from that study and is, therefore identified as the preferable point of departure for RfD derivation.

Uncertainty factor (UF) analysis - A **total UF of 1,000** was applied to the NOAEL based on the following individual UFs:

UF – study duration = 1
Although this was a less-than sub-chronic duration study, it appears appropriate to the relevant exposure scenarios.

UF – LOAEL-NOAEL = 1
The study yields a NOAEL.

UF – animal-human = 10
Standard assumption - this includes factors of 3 each for interspecies toxicokinetic and toxicodynamic variability.

UF – sensitive human populations = 10
Standard assumption– includes children as a sensitive group.

UF – database = 3
There is evidence from Astrachan et al. (1980) that anatoxin-a can cause developmental effects. However, that study does not yield a NOAEL. The Fawell and James (1994)/Fawell et al. (1999) study yields a NOAEL for developmental effects of 2,450 µg/kg/d (maternal dose). However, the method and data reporting for that portion of the study is inadequate. Thus, it is not entirely clear whether the NOAEL in the Fawell and James (1994)/Fawell et al. (1999) study is protective of developmental effects. In addition, there are no data that would allow an assessment of whether this NOAEL is protective of reproductive or immunotoxic effects.

UF – modifying factor = 3
The NOAEL from the critical study is less than an order of magnitude smaller than the LOAEL from the same study that reflects lethality.

UF-total = 1,000

RfD Calculation

RfD = NOAEL ÷ UF-total
= 98 µg/kg/d ÷ 1,000
= 0.098 µg/kg/d
which rounds to 0.1 µg/kg/d

Exposure Scenario - Water ingestion while swimming

Swimming is the most direct and pervasive activity that is likely to lead to exposure to hazardous algal toxins in surface water. Although there appears to be a potential for exposure to these algal toxins through inhalation and dermal exposure, direct ingestion appears to be the predominant

route of exposure while swimming (USEPA, 2016). Therefore, the exposure scenario is based on ingestion while swimming.

Water ingestion rate:

The USEPA Exposure Factors Handbook (EFH) (USEPA, 2011) provides guidance on incidental water ingestion while swimming. The rate of incidental water ingestion is greater (on both an absolute and body-weight adjusted basis) for children than for adults. For children (defined in the guidance as less than 18 years old), the guidance for the mean ingestion rate is 37 ml/event (45 min in the study used to derive this guidance) and 49 ml/hour. The upper percentile (reported as the 97th percentile of the distribution in the source study (Dufour et al., 2006) guidance for children is 90 ml/event (45 min) and 120 ml/hr. For episodic (as opposed to chronic) exposures, consistent with swimming events, the upper percentile values appear to be more appropriate. The EFH provides the value as generated by the recommended study based on the measurement time of 45 min. However, the EFH also provides a linear extrapolation of this value to a 1 hour swimming exposure in its recommendation. The length of a swimming event can be longer than the specific swimming event in the study used in the EFH, and a duration of one hour is assumed. It is recognized that the total recreational time spent near surface water used for swimming can be considerably longer than 1 hour. However, this duration of exposure refers specifically to time spent in the water.

Ingestion rate (upper percentile) for a 1 hour swimming event is 120 ml (0.12 L).

Frequency of exposure:

This is a difficult parameter to anticipate or model since it depends both on the frequency of swimming over the course of swimming season and the persistence of harmful algal blooms during that period. These appear to be highly variable. Rather than attempt to estimate this parameter quantitatively, the less-than sub-chronic to sub-chronic RfDs derived above will be assumed to be applicable as derived under the broad assumption that swimming events in water contaminated with toxins from harmful algal blooms can occur during multiple events over the course of the swimming season.

Body weight:

Because the source study used in the EFH to derive the recommended value for children's water ingestion (Dufour et al., 2006) does not specify the ages or age range of the children, the corresponding body weight cannot be derived directly from the recommended ingestion rate. The EFH provides recommendations for body weights for the entire range of childhood. Since hazardous algal blooms occur in natural waters (as opposed to, e.g., pools), it is assumed that an exposure scenario envisioning multiple swimming events would be most applicable to children who can swim and/or participate in water activities by themselves. The youngest age range addressed by the EFH that corresponds to this criterion is 6 to <11 years old. The mean body weight for this age group is given as 31.8 kg.

Body weight (ages 6 to <11 years) is 31.8 kg.

Calculation of recommended target concentrations of hazardous algal toxins

Equation

The recommended target water concentration of a toxin is given as:

$$C = \frac{RfD \times BW}{I}$$

Where:

C = the concentration of the toxin in the swimming water (μg/L, ppb)
RfD = the Reference Dose for the specific toxin (μg/kg-body wt/day)
BW = the assumed body weight of the child (31.8 kg)
I = the ingestion rate of swimming water (0.12 L/day)

Recommended target concentrations

Microcystin-LR
C = (0.01 μg/kg/d x 31.8 kg)/0.12 L/day = 2.65 μg/L
This is rounded to 3 μg/L.

Cylindrospermopsin
C = (0.03 μg/kg/d x 31.8 kg)/0.12 L/day = 7.95 μg/L
This is rounded to 8 μg/L.

Anatoxin-a
C = (0.1 μg/kg/d x 31.8 kg)/0.12 L/day = 26.5 μg/L
This is rounded to 27 μg/L.

Discussion

There are numerous uncertainties related to the recommended values for these hazardous algal toxins. The literature appropriate for consideration in RfD derivation was quite limited and in most cases only a single applicable study was available. The application of uncertainty factors of 1,000 (cylindrospermopsin and microcystin) and 1,000 (anatoxin-a) reflects the incompleteness of the databases for these toxins as well as the lack of clarity about the significance of reported outcomes. The specification of the likely exposure scenarios is highly uncertain due to lack of information about the nature, duration, and frequency of the actual exposures, and the likely duration of the toxins in any given waterbody. In particular, the density of blue-green algae at the surface of a body of water is subject to rapid change resulting from wind conditions. The uneven pattern of algal growth and the rapid shift in bloom density make obtaining a representative sample difficult.

Given these multiple uncertainties, the recommended water concentrations given here are intended to be protective of a range of exposures and are probably highly conservative (i.e., protective) for the most likely exposures. Nonetheless, the extent of this conservatism is not known. The uncertainty in the risk estimates as well as the inherent uncertainty in the temporal variability of the toxins in any given waterbody should be taken into account when considering advice to the public regarding recreation in affected waterbodies.

These recommendations do not address the risk to pets, livestock and wild fauna, nor do they address the risk associated with consuming fish from affected waters or the combined risk from swimming and fish consumption.

Citations

Astrachan, N.B.; 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 The Water Environment: Algal Toxins and Health; Carmichael, W.W., Ed.; Plenum Press: New York, NY, USA, 1981; pp. 437–446.

Astrachan, N.B., Archer, B.G., Hilbelink, D.R. (1980). Evaluation of the subacute toxicity and teratogenicity of anatoxin-a. Toxicon 18: 684-688.

Carmichael, W.W., Biggs, D.F., Gorham, P.R. (1975). Toxicology and pharmacological action of anabaena flos-aquae toxin. Science 187: 542-544.

Chen Y, Xu J, Li Y, Han X (2011). Decline of sperm quality and testicular function in male mice during chronic low-dose exposure to microcystin-LR. Reprod Toxicol. 31:551-7.

Chernoff N, Rogers EH, Zehr RD, Gage MI, et al. (2011). Toxicity and recovery in the pregnant mouse after gestational exposure to the cyanobacterial toxin, cylindrospermopsin. J Appl Toxicol. 31:242-54.

Chernoff, N., Rogers, E.H., Zehr, R.D., Gage, M.I., et al. (2014). The course of toxicity in the pregnant mouse after exposure to the cyanobacterial toxin cylindrospermopsin: clinical effects, serum chemistries, hematology, and histopathology. J. Toxicol. Environ. Health A 77:1040-60.

de Almeida CS, de Arruda ACC, de Queiroz EC, de Lima Costa HTM, Barbosa PF, Lemos TMAM, Oliveira CN, Pinto, E, Schwarz A, Kujbida P. Oral exposure to cylindrospermopsin in pregnant rats: reproduction and foetal toxicity studies. Toxicon 74:127-129, 2013.
Dufour, A.P., Evans, O., Behymer, T.D., Cantú, R. (2006). Water ingestion during swimming activities in a pool: a pilot study. J. Water Health 4:425-30.

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 and Experimental Toxicology, 18(3): 168-173.]

Fawell, J.K and James, H.A. (1994). Toxins from blue-green algae: toxicological assessment of anatoxin-a and a method for its determination in in reservoir water. Report no. FR 0492/DoE 3728 Aug. 1994. Foundation for Water Research, Buckinghamshire, UK

Fawell, J.K., James, C.P., James, H.A. (1994). Toxins from blue-green algae: toxicological assessment of microcystin-LR and a method for its determination in water. Report no. FR 0395/2/DoE 3358/2 Jan. 1994. Foundation for Water Research, Buckinghamshire, UK

Heinze, R. (1999). Toxicity of cyanobacterial toxin microcystin-LR to rats after 28 days intake with the drinking water. Environ. Toxicol. 14: 57-60.

Humpage, A.R., Falconer IR. (2003). Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: determination of no observed adverse effect level for deriving a drinking water guideline value. Environ. Toxicol. 18: 94-103.

Li XB, Zhang X, Ju J, Li Y, Yin L, Pu Y. (2014). Alterations in neurobehaviors and inflammation in hippocampus of rats induced by oral administration of microcystin-LR. Environ Sci Pollut Res Int 21:12419-25.

Li X, Zhang X, Ju J, Li Y, Yin L, Pu Y (2015). Maternal repeated oral exposure to microcystin-LR affects neurobehaviors in developing rats. Environ Toxicol Chem 34:64-9.

Lin, H., Liu, W., Zeng, H., Pu, C., et al. (2016). Determination of Environmental Exposure to Microcystin and Aflatoxin as a Risk for Renal Function Based on 5493 Rural People in Southwest China. Environ. Sci. Technol. 50: 5346-56.

Rogers, E. H., Zehr, R. D., Gage, M. I., et al. 2007. The cyanobacterial toxin, cylindrospermopsin, induces fetal toxicity in the mouse after exposure late in gestation. Toxicon, 49(6): 855-864.

Sukenik A, Reisner M, Carmeli S, Werman M (2006). Oral toxicity of the cyanobacterial toxin cylindrospermopsin in mice: long-term exposure to low doses. Environ Toxicol. 21:575-82.

Ueno, Y. Makita, Y., Nagata, S., Tsutsumi, T., et al. (1999). No chronic oral toxicity of a low dose of microcystin-LR, a cyanobacterial hepatotoxin, in female BALB/c mice. Environ. Toxicol. 14: 45-55.

USEPA (2011). Exposure Factors Handbook National Center for Environmental Assessment, Office of Research and Development EPA/600/R-09/052F September 2011 accessed at: file:///C:/Users/astern/Downloads/EFH-CHAPTER03.PDF, 7/5/16.

USEPA (2015a). Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins. Office of Water. Health and Ecological Criteria Division. EPA Document Number: 820R15100, June 15, 2015. Accessed at: https://www.epa.gov/sites/production/files/2015-06/documents/microcystins-report-2015.pdf, 7/8/16.

USEPA (2015b). Drinking Water Health Advisory for the Cyanobacterial Toxin Cylindrospermopsin. Office of Water. Health and Ecological Criteria Division. EPA Document Number: 820R15101, June 15, 2015. Accessed at: https://www.epa.gov/sites/production/files/2015-06/documents/cylindrospermopsin-report-2015.pdf, 7/11/16.

USEPA (2015c). Health Effects Support Document for the Cyanobacterial Toxin Microcystins. Office of Water. Health and Ecological Criteria Division, Washington, DC. EPA Document Number: 820R15102, June 15, 2015. Accessed at: https://www.epa.gov/sites/production/files/2015-06/documents/microcystins-support-report-2015.pdf.

USEPA (2015d). Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin. Office of Water. Health and Ecological Criteria Division, Washington, DC. EPA Document Number: 820R15103, June 15, 2015. Accessed at: https://www.epa.gov/sites/production/files/2015-06/documents/cylindrospermopsin-support-report-2015.pdf.

USEPA (2015e). Health Effects Support Document for the Cyanobacterial Toxin Anatoxin-A. Office of Water. Health and Ecological Criteria Division, Washington, DC. EPA Document Number: 820R15104 Date: June 15, 2015. Accessed at: https://www.epa.gov/sites/production/files/2015-06/documents/anatoxin-a-report-2015.pdf.

USEPA (2016). Draft Human Health Recreational Ambient Water Quality Criteria and/or Swimming Advisories for Microcystins and Cylindrospermopsin Documents. December 2016. https://www.epa.gov/wqc/draft-human-health-recreational-ambient-water-quality-criteria-andor-swimming-advisories

WHO (1998). World Health Organization. Cyanobacterial toxins: microcystin LR in drinking water. WHO/SDE/WSH/03.04/57.

WHO (2003a). World Health Organization. Guidelines for Safe Recreational Water Environments. Volume 1. Coastal and Fresh Waters. Chapter 8: Algae and Cyanobacteria in Fresh Water. Section 8.7, p. 149, and 8.8.1, p. 153. Geneva, Switzerland.

WHO (2003b). World Health Organization. Cyanobacterial toxins: Microcystin-LR in Drinking-water: Background document for development of WHO Guidelines for Drinking-water Quality WHO/SDE/WSH/03.04/57
Accessed at: http://www.who.int/water_sanitation_health/water-quality/guidelines/chemicals/cyanobactoxins.pdf?ua=1, 7/11/16.

Yavasoglu, A., Karaaslan, M.A., Uyanikgil, Y., Sayim, F., Ates, U., Yavasoglu, N.U. (2008). Toxic effects of anatoxin-a on testes and sperm counts of male mice. Exp. Toxicol. Pathol. 60:391-6.

Zhang XX, Zhang Z, Fu Z, Wang T, Qin W, Xu L, Cheng S, Yang L. (2010.)Stimulation effect of microcystin-LR on matrix metalloproteinase-2/-9 expression in mouse liver. Toxicol Lett. 2010 Dec 15;199(3):377-82.

Basis for NJDEP Reference Doses for Cyanotoxins

Gloria B. Post, Ph.D., DABT
Division of Science and Research
NJDEP
April 14, 2020

Summary

Recreational advisories for cyanotoxins are based on short-term Reference Doses. The scientific basis of the short-term Reference Doses for microcystin-LR, cylindrospermopsin and anatoxin-a were developed by the New Jersey Department of Environmental Protection (NJDEP) Division of Science and Research (DSR) in 2017 (NJDEP, 2017). The basis for these Reference Doses was reviewed in 2020 to determine whether any new information is available that would indicate updates are needed. Relevant recently published peer-reviewed studies were identified through a literature search and reviewed by DSR. This document provides the results of this review for these three cyanotoxins, including a comparison of the NJDEP and USEPA Reference Doses for microcystin-LR and cylindrospermopsin; there is no USEPA Reference Dose for anatoxin-a. Fifteen relevant new studies were identified and reviewed for microcystin-LR, one relevant new study was identified and reviewed for cylindrospermopsin, and no relevant new studies were identified for anatoxin-a. The new studies provide additional support for the Reference Doses of 0.01 µg/kg/day for microcystin, 0.03 µg/kg/day for cylindrospermopsin, and 0.1 µg/kg/day for anatoxin-a that were developed by DSR in 2017. As such, no updates to the Reference Doses are recommended at this time.

Introduction

New Jersey Department of Environmental Protection (NJDEP) Reference Doses used as the basis for recreational advisories for three cyanotoxins (microcystin-LR, cylindrospermopsin and anatoxin-a) were developed by the Division of Science and Research in 2017 (NJDEP, 2017) The basis of these Reference Doses was reviewed in 2020. The review included a literature search to identify relevant recently published peer-reviewed studies.

Recreational advisories for cyanotoxins are intended to be protective for children's swimming exposures during cyanobacteria harmful algal bloom (cyanoHAB) events, since children are the sensitive sub-population for swimming exposures. In New Jersey, cyanoHABs may persist for several months during the swimming season, and the recreational advisories are intended to protect for repeated daily exposures during the duration of a cyanoHAB event. Toxicity is considered through a short-term Reference Dose (µg/kg/day), which is the daily oral dose that is not expected to result in adverse health effects from short-term repeated exposures during a cyanoHAB event.

Process used to review basis of NJDEP (2017) Reference Doses

The PubMed database was searched 2015 through October 2019 for recent relevant studies that were not available when the NJDEP (2017) Reference Doses for microcystin-LR, cylindrospermopsin, and anatoxin-A were developed. These Reference Doses are intended to protect for repeated exposures to cyanotoxins during a cyanoHAB event. Relevant studies include repeated-dose oral (gavage or drinking water) studies with one of these cyanotoxins in mammalian species. Single-dose studies and studies in which dosing was via injection are not used as the primary basis for Reference Doses. Relevant recently published review articles were also considered, as discussed below.

Microcystin-LR Reference Dose

Basis of current NJDEP (2017) Reference Dose

The current NJDEP short-term Reference Dose (NJDEP, 2017) for microcystin-LR is 0.01 µg/kg/day. It is based on decreased weight gain and changes indicative of liver toxicity in mice dosed with microcystin-LR for 91 days (Fawell et al., 1994; 1999). The Lowest Observed Adverse Effect Level (LOAEL) in this study was 40 µg/kg/day, the lowest dose used, and a No Observed Adverse Effect Level (NOAEL) was not identified. Because the effects at 40 µg/kg/day were not severe, an uncertainty factor of 3, instead of the standard factor of 10, was used for LOAEL-to-NOAEL extrapolation.

As discussed in detail in NJDEP (2017), several other studies with durations applicable to development of a Reference Dose for recreational exposures to cyanotoxins during cyanoHAB events were reviewed, including short-term (>24 hours to 1 month) and subchronic (>1 month to 3 months) studies. These studies reported male reproductive, neurobehavioral and neurodevelopmental effects at doses (0.5 – 0.79 µg/kg/day) that are **50 to 80-fold lower** than 40 µg/kg/day, the LOAEL from Fawell et al. (1994; 1999). While these studies were not used as the primary basis for the Reference Dose because of issues related to their conduct and/or reporting, they suggested that **microcystin-LR causes toxicity at doses far below the dose** (40 µg/kg/day) used as the Point of Departure for the Reference Dose (NJDEP, 2017). As such, the NJDEP (2017) Reference Dose includes a database uncertainty factor of 10 to account for potentially more sensitive effects at lower doses.

Comparison with USEPA (2015a) Reference Dose

The USEPA (2015a) short-term Reference Dose for microcystin-LR is 0.05 µg/kg/day. It is based on a LOAEL of 50 µg/kg/day for liver toxicity (Heinze, 1999) that is very close to the LOAEL of 40 µg/kg/day for liver toxicity (Fawell et al., 1994/1999) used by NJDEP (2017).

The difference between the USEPA (2015a) and NJDEP (2017) Reference Doses is almost entirely due to the difference in the database uncertainty factor to account for data gaps and

potentially more sensitive effects. USEPA (2015a) also reviewed the studies mentioned above that showed effects at much lower doses, but used a partial database uncertainty factor of 3, while, for reasons discussed above, NJDEP (2017) used a full database uncertainty factor of 10. The other uncertainty factors used by USEPA are identical to those used by NJDEP (2017).

Additional recent toxicity studies

The PubMed literature search identified 15 peer-reviewed publications reporting on recent oral repeated-dose mammalian toxicity studies of microcystin-LR that were not considered by NJDEP (2017) or USEPA (2015a) in microcystin-LR Reference Dose development. In general, the newer studies do not have the methodological or reporting issues found in some of the older studies reporting effects at lower doses. These newer studies are summarized in Table 1.

The 15 publications included 6 studies in which the duration of exposure to microcystin-LR was 90 days or less. These studies are most relevant to development of short-term Reference Doses used as the basis for cyanotoxin recreational advisories. In all 6 of these studies, the LOAEL was lower than the LOAEL of 40 µg/kg/day used in the NJDEP (2017) Reference Dose, as summarized below. It should be noted that the doses in the drinking water studies are based on typical daily water consumption in mice of 0.2 ml/g/day[1] used to estimate the doses in several of the papers that were reviewed:

- He et al. (2017). Changes in metabolic profile and liver histology indicative of non-alcoholic fatty liver disease (NAFLD) occurred in mice after 90 days of exposure via gavage with a LOAEL of **20 µg/kg/day**. This was the lowest dose used, and a NOAEL was not identified.
- Lad et al. (2019). Biochemical and histological changes indicative of liver damage occurred in a strain of mice that is genetically modified to be a model for non-alcoholic fatty liver disease (NAFLD), after 28 days of gavage exposure with a LOAEL of **25 µg/kg/day**. This was the lowest dose used, and a NOAEL was not identified.
- Pan et al. (2018). Focal hyperplasia of the prostate occurred in mice after 90 days of drinking water exposure with a LOAEL of **2 µg/kg/day**. The NOAEL was 0.2 µg/kg/day. Effects were more severe after 180 days of exposure.
- Sedan et al. (2015). Lipid accumulation in the liver and decreased intraepithelial lymphocytes (immune system cells) in the small intestine occurred in mice after 30 days of gavage exposure with a LOAEL of **25 µg/kg/day**. This was the lowest dose used, and a NOAEL was not identified.
- Zhang et al. (2017). Effects on the reproductive system (decreased anogenital distance; decreased relative prostate weight, histopathological changes in the prostate, decreased

[1] The water consumption rate may have been lower than 0.2 ml/g/day based on estimates for mice provided by EFSA (2011) of 0.18 ml/g/day in subacute studies and 0.15 ml/g/day in subchronic study. If water consumption was lower than 0.2 ml/g/day, the doses (µg/kg/day) would also have been lower than those stated herein.

serum testosterone) occurred in 90-day old male offspring of female mice exposed through drinking water during pregnancy and lactation (12th day of gestation to 21st day after delivery). The LOAEL was **0.2 µg/kg/day**, the lowest dose used, with no NOAEL identified. Offspring were exposed prenatally and through breast milk, and potentially through direct access to drinking water at age 17-21 days.

- Zhou et al. (2020). An increase in the percentage of abnormal sperm tubules occurred in male mice after 90 days of drinking water exposure at a LOAEL of **20 µg/kg/day** and NOAEL of 2 µg/kg/day. With 180 days of exposure, the LOAEL was 2 µg/kg/day and the NOAEL was 0.2 µg/kg/day.

Nine additional studies with exposure durations longer than 90 days reported effects in mice at low doses including histological changes in the small intestine at 0.2 µg/kg/day (Cao et al., 2019), histopathological thyroid changes and decreased thyroid hormones at 2 µg/kg/day (Chen et al., 2019), histopathological changes in the lung at 1 µg/kg/day (Li et al., 2016), increased percent abnormal sperm at 1.5 µg/kg/day (Meng et al., 2019), decreased serum gonadotropin releasing hormone and testosterone at 1.5 µg/kg/day (Wang et al., 2018b), histopathological changes in the hippocampus at 1 µg/kg/day (Wang et al., 2018b), increased biochemical markers of brain inflammation at 1.5 µg/kg/day (Wang et al., 2019a), behavioral changes at 1.5 µg/kg/day (Wang et al., 2019b), and increased liver tumors at 2 µg/kg/day (Xu et al., 2018).

The occurrence of hepatic tumors in mice exposed to microcystin-LR in drinking water for one year (Xu et al, 2018) is particularly notable. Earlier studies (reviewed by WHO, 2003) showed that microcystin-LR can promote the growth of tumors after initiation with a genotoxic carcinogen. The only previous study of microcystin-LR as a complete carcinogen (Ito et al., 1997) found liver tumors after dosing by intraperitoneal injection but not after oral exposure; these results are not definitive because the study did not include an adequate control group and for other reasons. In contrast, Xu et al. (2018) is a well-conducted and well-reported study. Tumors were observed even although the dose groups were small (n=10), and tumor incidence increased in a generally dose-related manner (Control, 0.2 µg/kg/day, and 1 µg/kg/day – 0/10; 2 µg/kg/day – 1/10; 4 µg/kg/day – 3/10; 8 µg/kg/day – 2/10; 16 µg/kg/day – 4/10).

Additional considerations

Díez-Quijada et al. (2019a) reviewed the occurrence and toxicity of microcystin congeners other than microcystin-LR. They report that at least 246 forms of microcystin have been reported. They conclude that microcystin congeners other than microcystin-LR are distributed worldwide and may predominate, and that some of the other congeners may be more toxic than microcystin-LR. However, other forms of microcystin are not considered in the recreational advisory because most of the toxicological data on the effects of microcystins are for microcystin-LR, and other microcystin congeners are not analyzed in recreational waters. Because of the potential for co-exposure to other unidentified forms of microcystin of similar or greater toxicity, a public

health-protective approach is appropriate in development of the Reference Dose and recreational advisory for microcystin-LR.

Conclusions and Recommendations – Microcystin-LR Reference Dose

The recent studies reviewed above support the conclusion that the current NJDEP (2017) Reference Dose for microcystin-LR (0.01 µg/kg/day) is not overly conservative and that the full uncertainty factor of 10 to account for effects at lower doses than the Point of Departure is well-supported.

The LOAELs in all six shorter-term (subchronic or developmental) studies reviewed above and in Table 1 were below the LOAEL of 40 µg/kg/day used as the basis for the NJDEP (2017) Reference Dose. The LOAELs in the three gavage studies were 20-25 µg/kg/day (while noting that effects were reported in a mouse strain that is a model for NAFLD, but not in a comparable strain of wild-type mice in one of these studies).

Three additional drinking water studies found male reproductive effects (with evaluation of different specific endpoints in each study) at LOAELs of 0.2, 2, and 20 µg/kg/day, with the lowest LOAEL of 0.2 µg/kg/day from developmental exposure. The LOAELs of 0.2 and 2 µg/kg/day in two of these studies are 200- and 20-fold lower than the LOAEL (40 µg/kg/day) used for the current Reference Dose.

Finally, the recent longer-term studies provide additional evidence for a variety of toxic endpoints from low doses of microcystin-LR. Most notably, Xu et al. (2018) indicates that microcystin-LR can cause liver tumors in the absence of an initiator.

Based on the above information, no revision to the microcystin-LR Reference Dose of 0.01 µg/kg/day is recommended.

Cylindrospermopsin Reference Dose

Basis of current NJDEP (2017) Reference Dose

The current NJDEP short-term Reference Dose (NJDEP, 2017) for cylindrospermopsin is 0.03 µg/kg/day. It is based on the NOAEL of 30 µg/kg/day for increased relative kidney weight in mice exposed to cylindrospermopsin by gavage for 77 days; this effect occurred at doses of 60 µg/kg/day and above (Humpage and Falconer, 2003). There was no information that could be used to develop a Reference Dose for developmental or reproductive effects, and there was a lack of data on potential immune system and neurological effects. Because of these gaps in the toxicological database, a full uncertainty factor of 10 was applied, and the total uncertainty factor was 1000.

Comparison with USEPA (2015b) Reference Dose

The USEPA (2015b) short-term Reference Dose for cylindrospermopsin is 0.1 µg/kg/day. It is based on the same NOAEL for increased relative kidney weight (Humpage and Falconer, 2003) as the NJDEP (2017) Reference Dose. The difference between the USEPA (2015b) and NJDEP (2017) Reference Doses is due to the difference in the database uncertainty factor to account for data gaps and potentially more sensitive effects. USEPA (2015b) used a partial database uncertainty factor of 3, while, for reasons discussed above, NJDEP (2017) used a full database uncertainty factor of 10.

Additional recent toxicity studies

The PubMed literature search identified only one new study (Díez-Quijada et al., 2019b) of the toxicity of cylindrospermopsin. In this study, male Wistar rats were administered a single dose of 0, 7.5, 23.7, or 75 µg/kg by gavage. It should be noted that the other toxicity studies of cylindrospermopsin reviewed by NJDEP (2017) were conducted in mice, with the exception of one rat study (de Almeida et al., 2013) that used lower doses than Díez-Quijada et al. (2019b). Genotoxicity was evaluated in bone marrow with the micronucleus test and in the comet assay in stomach, liver, and blood, and histopathological examinations were performed on stomach and liver. The percent of micronuclei in bone marrow cells was increased at all doses compared to the controls, but this effect did not increase with increasing dose. The authors state that these positive results in an *in vivo* study confirm earlier reports of *in vitro* genotoxicity. In contrast, the comet assay for DNA strand breaks was negative at all doses in blood, stomach, and liver. Histopathological changes, with severity increasing with dose, were observed in livers and stomachs at all dose levels.

Conclusions and Recommendations for Cylindrospermopsin Reference Dose

The recent study (Díez-Quijada et al., 2019b) reviewed above supports the conclusion that the current NJDEP (2017) Reference Dose for cylindrospermopsin (0.03 µg/kg/day) is not overly conservative and that the full database uncertainty factor of 10 to account for effects at lower doses than the Point of Departure is well-supported. Genotoxicity and histopathological changes were observed from a single exposure to 7.5 µg/kg. This dose is 4-fold lower than the NOAEL of 30 µg/kg/day and 8-fold lower than the LOAEL of 60 µg/kg/day in the 77-day study used as the basis for the current NJDEP Reference Dose.

Based on the above information, no revision to the cylindrospermopsin Reference Dose of 0.03 µg/kg/day is recommended.

Anatoxin-a Reference Dose

Basis of current NJDEP (2017) Reference Dose

The current NJDEP short-term Reference Dose (NJDEP, 2017) for anatoxin-a is 0.1 µg/kg/day. It is based on the NOAEL for lethality of 98 µg/kg/day in mice exposed to anatoxin-a by gavage for 28 days (Fawell and James, 1994; Fawell et al., 1999). The Reference Dose includes a total uncertainty factor of 1000 including a factor of 3 for database gaps regarding developmental, reproductive, and immune system effects and a modifying factor of 3 because the NOAEL is less than 10-fold lower than the LOAEL for lethality in the same study.

Comparison with USEPA (2015c) Reference Dose

The USEPA (2015c) concluded that there are insufficient data to derive a Reference Dose for anatoxin-a at this time.

Additional Recent Toxicity Studies

The PubMed literature search identified no toxicity studies that were not considered by NJDEP (2017).

Conclusions and Recommendation for Anatoxin-a Reference Dose

No revision to the current Reference Dose of 0.1 µg/kg/day is recommended.

CITATIONS

Cao L, Huang F, Massey IY, Wen C, Zheng S, Xu S, Yang F (2019). Effects of Microcystin-LR on the Microstructure and Inflammation-Related Factors of Jejunum in Mice. Toxins (Basel). 11(9).

Chen J, Bian R, Li J, Qiu L, Lu B, Ouyang X (2019). Chronic exposure to microcystin-LR reduces thyroid hormone levels by activating p38/MAPK and MEK/ERK signal pathway. Ecotoxicol Environ Saf. 173:142-148.

Díez-Quijada L, Prieto AI, Guzmán-Guillén R, Jos A, Cameán AM. (2019a). Occurrence and toxicity of microcystin congeners other than MC-LR and MC-RR: A review. Food Chem Toxicol. 125:106-132.

Díez-Quijada L, Llana-Ruiz-Cabello M, Cătunescu GM, Puerto M, Moyano R, Jos A, Cameán AM. (2019b). In vivo genotoxicity evaluation of cylindrospermopsin in rats using a combined micronucleus and comet assay. Food Chem Toxicol. 132:110664.

EFSA (2011). European Food Safety Authority. Guidance on Default assumptions used by the EFSA Scientific Panels and Committee, and EFSA Units in the absence of actual measured data. DRAFT guidance on Default assumptions.
https://www.efsa.europa.eu/sites/default/files/consultation/110707a.pdf

Fawell, J.K and James, H.A. (1994). Toxins from blue-green algae: toxicological assessment of anatoxin-a and a method for its determination in in reservoir water. Report no. FR 0492/DoE 3728 Aug. 1994. Foundation for Water Research, Buckinghamshire, UK.

Fawell, J.K., James, C.P., James, H.A. (1994). Toxins from blue-green algae: toxicological assessment of microcystin-LR and a method for its determination in water. Report no. FR 0395/2/DoE 3358/2 Jan. 1994. Foundation for Water Research, Buckinghamshire, UK

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 and Experimental Toxicology, 18(3): 168-173.

He J, Li G, Chen J, Lin J, Zeng C, Chen J, Deng J, Xie P (2017).. Prolonged exposure to low-dose microcystin induces nonalcoholic steatohepatitis in mice: a systems toxicology study. Arch Toxicol. 91:465-480.

Heinze, R. (1999). Toxicity of cyanobacterial toxin microcystin-LR to rats after 28 days intake with the drinking water. Environ. Toxicol. 14: 57-60.

Humpage, A.R., Falconer IR. (2003). Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: determination of no observed adverse effect level for deriving a drinking water guideline value. Environ. Toxicol. 18: 94-103.

Lad A, Su RC, Breidenbach JD, Stemmer PM, Carruthers NJ, Sanchez NK, Khalaf FK, Zhang S, Kleinhenz AL, Dube P, Mohammed CJ, Westrick JA, Crawford EL, Palagama D, Baliu-Rodriguez D, Isailovic D, Levison B, Modyanov N, Gohara AF, Malhotra D, Haller ST, Kennedy DJ (2019). Chronic Low Dose Oral Exposure to Microcystin-LR Exacerbates Hepatic Injury in a Murine Model of Non-Alcoholic Fatty Liver Disease. Toxins (Basel). 11(9).

Li X, Xu L, Zhou W, Zhao Q, Wang Y (2016). Chronic exposure to microcystin-LR affected mitochondrial DNA maintenance and caused pathological changes of lung tissue in mice. Environ Pollut. 210:48-56.

Meng X, Peng H, Ding Y, Zhang L, Yang J, Han X (2019). A transcriptomic regulatory network among miRNAs, piRNAs, circRNAs, lncRNAs and mRNAs regulates microcystin-leucine arginine (MC-LR)-induced male reproductive toxicity. Sci Total Environ. 667:563-577.

NJDEP (2017). Recommended NJ Action Level and Health Advisory Guidelines for Recreational Exposure to Microcystin-LR, Cylindrospermopsin, and Anatoxin-A. Appendix I of NJDEP (2018). Cyanobacterial Harmful Algal Bloom (HABs) Freshwater Recreational Response Strategy.

Pan C, Chen Y, Xu T, Wang J, Li D, Han X (2018). Chronic exposure to microcystin-leucine-arginine promoted proliferation of prostate epithelial cells resulting in benign prostatic hyperplasia. Environ Pollut. 242 (Pt B):1535-1545.

Sedan D, Laguens M, Copparoni G, Aranda JO, Giannuzzi L, Marra CA, Andrinolo D (2015). Hepatic and intestine alterations in mice after prolonged exposure to low oral doses of Microcystin-LR. Toxicon. 104:26-33.

USEPA (2015a). Health Effects Support Document for the Cyanobacterial Toxin Microcystins. Office of Water. EPA- 820R15102. June 2015.

USEPA (2015b). Health Effects Support Document for the Cyanobacterial Toxin Cylindrospermopsin. Office of Water. EPA- 820R15103. June 2015.

USEPA (2015c). Health Effects Support Document for the Cyanobacterial Toxin Anatoxin-A. Office of Water. EPA- 820R15104. June 2015.

Wang J, Chen Y, Chen Z, Xiang Z, Ding J, Han X (2018a). Microcystin-leucine arginine inhibits gonadotropin-releasing hormone synthesis in mice hypothalamus. Ecotoxicol Environ Saf. 2018 Nov 15; 163:391-399. Office of Water. EPA 822-R-19-001. May 2019.

Wang X, Xu L, Li X, Chen J, Zhou W, Sun J, Wang Y (2018b). The differential effects of microcystin-LR on mitochondrial DNA in the hippocampus and cerebral cortex. Environ Pollut. 2018 Sep; 240:68-76.

Wang J, Zhang C, Zhu J, Ding J, Chen Y, Han X (2019a). Blood-brain barrier disruption and inflammation reaction in mice after chronic exposure to Microcystin-LR. Sci Total Environ. 689: 662-678.

Wang J, Chen Y, Zhang C, Xiang Z, Ding J, Han X (2019b). Learning and memory deficits and Alzheimer's disease-like changes in mice after chronic exposure to microcystin-LR. J Hazard Mater. 373:504-518.

Xu L, Li T, Ding W, Cao Y, Ge X, Wang Y (2018). Combined seven miRNAs for early hepatocellular carcinoma detection with chronic low-dose exposure to microcystin-LR in mice. Sci Total Environ. 628-629:271-281.

Zhang H, Wang L, Shen S, Wang C, Xiang Z, Han X, Li D (2017). Toxic effects of microcystin-LR on the development of prostate in mice. Toxicology 380:50-61.

Zhou Y, Sun M, Tang Y, Chen Y, Zhu C, Yang Y, Wang C, Yu G, Tang Z (2020). Responses of the proteome in testis of mice exposed chronically to environmentally relevant concentrations of Microcystin-LR. Ecotoxicol Environ Saf. 187:109824.

Table 1: Recently Published Repeated-Dose Oral Toxicity Studies of Microcystin-LR

Study	*Species, Sex, Strain*	*Duration*	*Exposure Route*	*Exposure Levels*	*Doses* (µg/kg/day)*	*Most sensitive effect(s)*	*NOAEL (µg/kg/day)*	*LOAEL (µg/kg/day)*	*Comments*
Cao et al., 2019	Mouse (sex not specified) - C57Bl/6J	180 days	Drinking Water	0, 1, 30, 60, 90, 120 µg/L	0, 0.2, 6, 12, 18, 24	Histological changes in jejunum (small intestine)	----	0.2	
Chen et al., 2019	Mouse (female) - Balb/c	180 days	Drinking Water	0, 1, 10, 20, 40 µg/L	0, 0.2, 2, 4, 8	Decreased thyroid hormone FT3; increased % apoptotic cells in thyroid	0.2	2	Changes in related hormones (FT4; TSH) at higher doses
He et al., 2017	Mouse (male) - Balb/c	**90 days**	Gavage	0, 40, 200 µg/kg every 2 days	0, 20, 100	Changes in metabolite profiles and liver histology indicative of non-alcoholic steatosis (i.e. non-alcoholic fatty liver disease - NAFLD)	---	**20**	Subchronic study with LOAEL below 40 µg/kg/day used in NJDEP (2016)
Lad et al., 2019	Mouse (male) - Ledpr^{db} (NAFLD model strain); C57Bl/6J (control strain)	**28 days**	Gavage	0, 50, 100 µg/kg every 2 days	0, 25, 50	Biochemical and histological markers of liver damage in Ledpr^{db} strain; no effects in C57Bl/6J	**--- Ledpr^{db}** **50 - C57Bl/6J**	**25 - Ledpr^{db}** **--- C57Bl/6J**	NAFLD increases susceptibility to hepatic toxicity of microcystin-LR Differing results than He et al. (2017) in control strain may be due to differing susceptibility to hepatic toxicity in Balb/c versus C57Bl/6J strains and/or the longer duration of exposure in He et al. (2017)
Li et al., 2016	Mouse (male) – C57BL/6	360 days	Drinking Water	0, 1, 5, 10, 20, 40 µg/L	0, 0.2, 1, 2, 4, 8	Histopathological changes in the lung	0.2	1	

Study	*Species, Sex, Strain*	*Duration*	*Exposure Route*	*Exposure Levels*	*Doses* (µg/kg/day)*	*Most sensitive effect(s)*	*NOAEL (µg/kg/day)*	*LOAEL (µg/kg/day)*	*Comments*
Meng et al., 2019	Mouse (male) – Balb/c	180 days	Drinking Water	0, 1, 7.5, 15, 30 µg/L	0, 0.2, 1.5, 3, 6	% abnormal sperm	0.2	1.5	LOAEL for decreased relative testes wt. – 3 µg/kg/day
Pan et al., 2018	Mouse (male) – Balb/c	**90** and 180 days	Drinking Water	0, 1, 10, 20, 30 µg/L	0, 0.2, 2, 4, 6	Focal hyperplasia of prostate (90 days); more severe changes at 180 days.	**0.2**	**2**	Dose-related increase in biochemical markers of prostate disease
Sedan et al. 2015	Mouse (male) – N:NIH-S	**30 days**	Gavage	0, 50, 100 µg/kg every 2 days	0, 25, 50	Hepatic steatosis (lipid accumulation) and decreased intraepithelial lymphocytes in small intestine	---	**25**	Intraepithelial lymphocytes are involved with immune response in small intestine
Wang et al., 2018a	Mouse (male) – ICR	180 days	Drinking Water	0, 1, 7.5, 15, 30 µg/L	0, 0.2, 1.5, 3, 6	Decreased serum gonadotropin releasing hormone (GnRH) and testosterone	0.2	1.5	Decreases were dose-related; non-significant decreases at 0.2 µg/kg/day
Wang et al., 2018b	Mouse (male) – C57BL/6	360 days	Drinking Water	0, 1, 5, 10, 20, 40 µg/L	0, 0.2, 1, 2, 4, 8	Histopathological changes and effects on mitochondrial DNA in hippocampus and cerebral cortex, more severe in hippocampus	--	1	Severity of histopathological changes in hippocampus increased with dose
Wang et al., 2019a	Mouse (male) – Balb/c	180 days	Drinking Water	0, 1, 7.5, 15, 30 µg/L	0, 0.2, 1.5, 3, 6	Increased glial fibrillary acidic protein (GFAP; marker of astrocyte activation) and TNF-alpha	0.2	1.5	Additional conclusions – microcystin-LR impaired blood-brain barrier and accumulated in mouse brain

						(marker of brain inflammation) in brain			
Study	***Species, Sex, Strain***	***Duration***	***Exposure Route***	***Exposure Levels***	***Doses* (µg/kg/day)***	***Most sensitive effect(s)***	***NOAEL (µg/kg/day)***	***LOAEL (µg/kg/day)***	***Comments***
Wang et al., 2019b	Mouse (sex not specified) - Balb/c	180 days	Drinking Water	0, 1, 7.5, 15, 30 µg/L	0, 0.2, 1.5, 3, 6	Decreased scores on tests of cognitive impairment – "freezing time" in context test, and novel object recognition test	0.2	1.5	Other changes characteristic of Alzheimer's disease including effects on learning and memory, and histological and biochemical changes in the brain.
Xu et al., 2018	Mouse (male) – C57BL/6	90, 180, 270, 360 days	Drinking Water	0, 1, 5, 10, 20, 40, 80 µg/L	0, 0.2, 1, 2, 4, 8, 16	Liver tumors after 360 days exposure. Atypical liver cells after 270 days exposure	1	2	No statistical analysis shown for tumor incidence, but no tumors in controls or lower dose groups. An earlier study (Ito et al., 1997) showing that microcystin-LR caused liver tumors used intraperitoneal injection and did not include a control group.
Zhang et al., 2017	Mouse (male offspring) – Balb/c	**Maternal exposure from gestational day 12 to postnatal day (PND) 21 (~ 29 total days of exposure)** Offspring received *in utero* and	Drinking Water	0, 1, 10, 50 µg/L	0, 0.2, 2, 10 (estimated maternal dose) Potential doses to offspring via ingestion of drinking	In offspring at 90 days of age: Decreased anogenital distance; decreased body wt., decreased relative prostate wt., histopathological changes in the prostate, decreased serum testosterone	----	**0.2**	The effects were clear cut in the lowest dose group (maternal - 0.2 µg/kg/day). LOAEL for effects in offspring at 30 days of age was (maternal) dose of 2 µg/kg/day. It was noted that offspring may have ingested the drinking water directly

		lactational exposure, and possibly exposure via ingestion of drinking water on PND 17-21.			water on PND 17-21 are not known.				on PND 17-21, and that the dose received from drinking water ingestion may have been substantial compared to *in utero* and lactation exposure.
Study	***Species, Sex, Strain***	***Duration***	***Exposure Route***	***Exposure Levels***	***Doses* (µg/kg/day)***	***Most sensitive effect(s)***	***NOAEL (µg/kg/day)***	***LOAEL (µg/kg/day)***	***Comments***
Zhou et al., 2020	Mouse (male), strain not specified	**90** and 180 days	Drinking Water	0, 1, 10, 100 µg/L	0, 0.2, 2, 20	% abnormal sperm tubules	**2 – 90 day exposure** 0.2 – 180 day exposure	**20 – 90 day exposure** 2 – 180 day exposure	Changes in relevant biochemical markers were evaluated at 180 days and were affected by microcystin-LR

NOTE: Study durations, LOAELs and NOAELs from studies with durations of **90 days or less** are shown in **BOLD UNDERLINE** because they are most relevant to development of the short-term Reference Doses used in recreational advisories.

*Doses (µg/kg/day) in studies using drinking water exposure are estimated based on a daily water consumption rate in mice of 0.2 ml/g/day used to estimate the doses in several of the papers that were reviewed. The water consumption rate may have been lower than 0.2 ml/g/day based on estimates for mice provided by EFSA (2011) of 0.18 ml/g/day in subacute studies and 0.15 ml/g/day in subchronic study. If water consumption was lower than 0.2 ml/g/day, the doses (µg/kg/day) would also have been lower than those stated herein.

Basis for NJDEP Recreational Advisory for Saxitoxin

Brian Pachkowski, Ph.D.
Division of Science and Research
February 2021

Executive summary

Saxitoxin (STX) is a member and the representative molecule of a class (i.e., the saxitoxins) of over 50 structurally related analogues produced by cyanobacteria in freshwater environments. During cyanobacteria harmful algal bloom (cyanoHAB) events, humans can be exposed to STX and its analogues through recreational activities (e.g., incidental ingestion of water during swimming). The Division of Science and Research developed the scientific basis of the NJDEP recreational advisory for STX. The short-term oral reference dose (RfD) and recreational advisory derived here are intended to be protective for oral exposure on multiple days of swimming during the swimming season, for the more sensitive sub-population of children.

Neurotoxicity is the major health effect in humans and laboratory animals, particularly following acute oral exposure. The ability of STX to cause other health effects (e.g., systemic, developmental, reproductive, or immune toxicity) after either acute or prolonged exposure is generally unknown, as such effects have not been as thoroughly studied.

The limited number of studies in laboratory animals that demonstrate the ability of STX to cause neurotoxicity were judged not appropriate for the derivation of a short-term STX RfD (e.g., inadequate study design or data reporting, potential co-exposure to other bacterial toxins, assessment of only sub-clinical endpoints). A number of assessments have reviewed case reports of paralytic shellfish poisoning (PSP) in humans, which is caused by STX and its analogues. Of these assessments, Arnich and Thébault (2018) is deemed most scientifically appropriate for deriving a short-term RfD and recreational guidance value for STX, because of the systematic review approach used to identify and assess relevant data, subsequent statistical modeling of PSP data, and peer-review.

In modeling the human PSP data, Arnich and Thébault (2018) derived a point of departure (POD) of 0.37 µg STX/kg. A composite uncertainty factor of 100, which accounts for human variability (factor of 3), the use of acute PSP exposure data for the derivation of a short-term RfD (factor of 3), and database deficiencies (factor of 10 for lack of developmental, reproductive, and immune studies), was applied to the POD yielding a short-term STX RfD of 0.0037 µg/kg/day.

Based on the assumed body weight of a child (31.8 kg) and the daily incidental ingestion rate of swimming water (0.21 L/day) from the USEPA (2019), an STX recreational guidance value of 0.6 µg/L is derived.

The USEPA does not have an RfD or recreational exposure guidance value for STX. However, five US states (CO, OH, OR, PA, WA) have recreational water guideline levels for STX. All are based on the same principal study (EFSA, 2009) and critical effect (PSP in humans). Using 0.5 µg/kg/day as a POD, these states applied additional UFs (e.g., for human variability or database

limitations) to derive acute or short-term RfDs. The states used these RfDs and relevant exposure factors to derive their recreational values, which range from 0.8 to 75 µg/L. Of these values, only the OH EPA and PA DEP value of 0.8 µg/L is close to the NJDEP value, while the other states' values (4 to 75 µg/L) are higher.

In summary, an STX recreational guidance value of 0.6 µg/L was derived and is recommended for use during New Jersey cyanoHAB events.

Introduction

At the request of the New Jersey Department of Environmental Protection's (NJDEP) Bureau of Freshwater and Biological Monitoring, the scientific basis of the NJDEP recreational advisory for saxitoxin (STX) was developed by the Division of Science and Research (DSR).

Recreational advisories for cyanotoxins are intended to be protective for children's swimming exposures during cyanobacteria harmful algal bloom (cyanoHAB) events, since children are the sensitive sub-population for swimming exposures. In New Jersey, cyanoHABs may persist for several months during the swimming season, and the recreational advisories are intended to protect for repeated daily exposures during the duration of a cyanoHAB event (USEPA, 2019; NJDEP, 2020).

These recreational advisories (µg/L) are based on both toxicity and exposure considerations:

- Toxicity is considered through a short-term Reference Dose (RfD; µg/kg/day), which is the daily oral dose that is not expected to result in adverse health effects from short-term exposures.
- The exposure pathway of concern is incidental ingestion of water by children while swimming. The exposure factors used are the amount of water swallowed per day by a child during swimming (L/day) and the child's body weight (kg).

The bases for both the STX short-term RfD and the exposure assumptions used to develop the advisory are discussed below.

Document development process

Literature searches were conducted by the Department's Environmental Research Library on April 2019 and February 2020 to identify resources to inform the derivation of an RfD for a recreational guidance value for STX. These searches were supplemented by relevant literature identified in the reference sections of authoritative sources (e.g., government and health agency reports) and review articles. In addition to internal NJDEP review, the scientific basis of the NJDEP RfD for STX described herein underwent review by three external peer-reviewers.

Background information relevant to health effects of STX

STX is a member and the representative molecule of a class (i.e., the saxitoxins) of over 50 structurally related analogues (e.g., neosaxitoxin, gonyautoxins). These naturally occurring toxins are hydrophilic and not volatile (Testai et al., 2016; Vilariño et al., 2018; WHO, 2020). STX is considered to be heat (even at 100°C) and acid stable but is unstable under alkaline conditions (EFSA, 2009). In the environment, STX has been shown to persist for up to 2 months in water (WHO, 2020). However, in laboratory experiments, some bacteria have been shown to degrade STX and its analogues within a short period of time (< 3 days) and transform one analogue to another (Donovan et al., 2008; Smith et al., 2001). Further in-depth information regarding the structure and chemical and physical properties of STX can be found elsewhere (WHO, 2020).

Occurrence and human exposure to STX

In freshwater environments, cyanobacteria produce STX and its analogues, whereas dinoflagellates generally produce these toxins in marine environments and brackish waters (WHO, 2020).

The oral route is the main route of human exposure to STX. During cyanoHAB events in freshwater, humans can be exposed to STX and its analogues through recreational activities (e.g., incidental ingestion of water during swimming) and/or drinking water, particularly where drinking water treatment is insufficient or non-existent (WHO, 2020). Additionally, the consumption of marine shellfish contaminated with STX and its analogues (i.e., from feeding on toxin-producing prey) is a well-known route of human oral exposure (Testai et al., 2016).

Although not volatile, inhalation exposure could potentially occur if STX was present in aerosols (e.g., resulting from the wake of a boat) (WHO, 2020). While dermal exposure to STX may occur during recreational activities, dermal absorption is unlikely (WHO, 2020). STX does not appear to irritate or sensitize the eye or skin (except for tingling or numbness of the lips) (WHO, 2020).

Toxicokinetics of STX

Information on the human toxicokinetics (i.e., absorption, distribution, metabolism, and excretion) of STX has largely been ascertained following episodes of human ingestion of shellfish contaminated with STX and its analogues. Toxicokinetic studies in other mammalian models (e.g., cats) are reviewed elsewhere (EFSA, 2009; WHO, 2020).

The absorption of STX and its analogues at the point of contact (i.e., lips, mouth, tongue) and the gastrointestinal tract is efficient, as symptoms occur minutes to hours following oral exposure in humans (EFSA, 2009; WHO, 2020).

STX and its analogues are distributed throughout the human body. Post-mortem analyses of individuals who had died from paralytic shellfish poisoning (PSP), which is caused by STX and its analogues (EFSA, 2009), demonstrated that these toxins were present in the adrenal glands, bile, brain, cerebrospinal fluid, heart, kidneys, liver, lungs, pancreas, spleen, and thyroid gland

(Vilariño et al., 2018). Following intraperitoneal exposure in pregnant mice, STX was reported to cross the placental barrier and reach the fetal brain (Lima-Filho et al., 2020).

The human metabolism of STX has not been clearly elucidated. However, post-mortem analyses of PSP victims suggest that STX and its analogues undergo metabolism, as toxin profiles of the victims' gastric contents differ from the profile in other specimens (e.g., urine, liver, kidneys) (Vilariño et al., 2018). Using human liver microsomes, *in vitro* investigations suggest that STX can undergo N-oxidation and glucuronidation reactions (WHO, 2020). The N-oxidation of STX leads to the formation of neosaxitoxin, which itself is capable of producing toxicity (Testai et al., 2016). In addition to oxidation, other metabolic reactions (e.g., hydrolysis, sulfation) lead to other STX biotransformation products (WHO, 2020).

Urine is the major route of elimination for STX and its analogues in humans, although these toxins have also been detecting in bile suggesting a fecal route of elimination (WHO, 2020). STX appears to be eliminated from the human body relatively rapidly. Based on individuals recovering from PSP, STX and its analogues were cleared from the serum to undetectable levels within 24 hours of exposure, and a human serum half-life of less than 10 hours was estimated (Gessner et al., 1997). A urinary human half-life of 20.4 hours has also been reported (Wharton et al., 2017). This relatively short half-life is supported by studies in laboratory rats that reported half-lives between 12 and 18 hours following intravenous injection with either STX or a reduced derivative, saxitoxinol (EFSA, 2009).

Human and laboratory animal health effects of STX

As reviewed below, neurotoxicity is the major health effect in humans and laboratory animals (e.g., rodents) following acute oral exposure to STX (EFSA, 2009). Due to a lack of information, the human and laboratory animal health effects from chronic oral exposure to STX are not definitively known. Health effect studies in other species (e.g., zebrafish) are reviewed elsewhere (O'Neill et al., 2016; Testai et al., 2016).

<u>Acute effects in humans</u>

The acute human health effects of STX have been identified from observations in individuals who consumed shellfish contaminated with STX and its analogues, which lead to PSP (EFSA, 2009). Because of their causative role in PSP, STX and its analogues have been called paralytic shellfish toxins (Vilariño et al., 2018). PSP is a collection of acute neurological symptoms of various severities: mild (e.g., tingling or numbness around the mouth or digits, headache, dizziness, nausea, vomiting); moderate (e.g., numbness and weakness in extremities, ataxia, incoherent speech, shortness of breath); and severe (e.g., muscular paralysis, respiratory difficulties). Death can also result from respiratory paralysis (WHO, 2020). No antidote is available for PSP (Testai et al., 2016). No data were identified on whether acute STX exposure causes effects other than neurotoxicity (e.g., systemic, developmental, reproductive, or immune effects) in humans (WHO, 2020).

<u>Acute effects in animals</u>

As discussed below in the "Derivation of an STX RfD" section, a limited database exists for the acute effects of oral STX exposure in laboratory animals. In general, such studies have focused

on and confirmed the neurotoxicity of STX. Aside from neurotoxicity, the potential for STX to cause overt acute toxicity has not be evaluated.

Chronic effects in humans

No studies have been identified that investigated human health effects from chronic exposure to STX (WHO, 2020). However, there is speculation that low dose STX exposure during different stages of human development may cause long-term, permanent effects. For example, STX exposure during neurogenesis may affect neurodevelopment, since STX interacts with ion channels on neuronal cells and may thereby inhibit the cellular electrical activity that occurs during neurodevelopment (O'Neill et al., 2016).

Subchronic and chronic effects in animals

There are limited chronic data (i.e., those with at least 90 days of exposure) regarding the effects of STX in laboratory animals. However, as reviewed below, subchronic (~30 days) studies in rats exposed to drinking water containing cyanobacterial cultures producing STX and its analogues confirm the neurotoxicity of STX. With the exception of biochemical changes in the liver that were evaluated in one study, these studies did not assess toxicological endpoints other than neurotoxicity.

The study with the longest duration of exposure involved male rats exposed to neosaxitoxin, which is an analogue and metabolite of STX, for 12 weeks via daily subcutaneous injection (Zepeda et al., 2014). Only rats in the high dose group (6 µg/kg/day) exhibited signs of toxicity, including increases in total and direct bilirubin, gamma-glutamyltransferase, and serum glutamic oxaloacetic transaminase, which indicate impaired liver function. These effects were reversible following cessation of exposure. No other signs of toxicity were observed in terms of body weight, food intake, hematological and biochemical parameters, and organ weight and histopathology (heart, kidney, liver, lung, spleen, and stomach).

Mode of action

The neurotoxicity of STX results from its ability to bind to voltage-gated sodium channels (VGSCs) on neuronal cells (EFSA, 2009; O'Neill et al., 2016; WHO, 2020). Specifically, STX binds to site 1 of the α-subunit of the VGSCs found on the outside of these cells. In doing so, STX blocks these channels thereby preventing sodium ions from moving across the neuronal cell membrane. Blocking the movement of sodium ions prevents the generation of action potentials along neuronal axons and the transmission of nerve impulses to muscles. A progressive loss of neuromuscular function occurs resulting in paralytic symptoms that may ultimately lead to death by respiratory arrest. This mode of action is believed to be consistent for most, if not all, of the different STX analogues (Testai et al., 2016).

Humans have 10 different isoforms or variations of the α-subunit of VGSCs (O'Neill et al., 2016; WHO, 2020). The distribution of these isoforms can vary throughout the human body (i.e., some may occur predominantly in the central or peripheral nervous systems) and their expression can vary during development. Additionally, each isoform may have a different sensitivity toward STX. Such differences in distribution, expression, and sensitivity may be an explanation for why some individuals are more susceptible to STX.

In addition to causing toxicity by binding to VGSCs, STX is reported to bind to calcium and potassium channels. Inhibition of these channels, which may result in toxicity, appears to occur with much higher STX doses compared to the inhibition of VGSCs (O'Neill et al., 2016; WHO, 2020). In an *in vitro* mouse model, effects on cellular proliferation and differentiation is suggestive of STX binding to voltage-gated calcium channels, which may have implications for neurodevelopment (Lima-Filho et al., 2020). Oxidative stress may also result from STX exposure; however, this may be more relevant to longer durations of STX exposure (O'Neill et al., 2016; WHO, 2020).

STX recreational values used by other states

As of August 2020, the USEPA does not have a toxicity value (e.g., RfD) or recreational exposure guidance value for STX. However, five US states (CO, OH, OR, PA, WA) are reported by USEPA (2019) to have recreational water guideline levels for STX. While all are based on the same principal study (EFSA, 2009), these recreational values range from 0.8 to 75 µg/L (Appendix A).

As reviewed in detail in the "Human epidemiology studies" section below, the EFSA (2009) assessment summarized human case reports (including > 500 individuals) of paralytic shellfish poisoning (PSP), for which STX and its analogues were the causative toxins (EFSA, 2009). EFSA (2009) estimated a lowest-observed-adverse-effect-level (LOAEL) of 1.5 µg STX equivalents/kg/day (or µg STX eq/kg/day)[1].

EFSA (2009) applied an uncertainty factor (UF) of 3 to the LOAEL of 1.5 µg/kg/day to estimate a no-observed-adverse-effect level (NOAEL) of 0.5 µg /kg/day. Using 0.5 µg /kg/day as a point of departure (POD), the states listed above then applied additional UFs (e.g., for human variability or database limitations) to derive acute or short-term RfDs. The states used these RfDs and relevant exposure factors (e.g., body weight, incidental water ingestion) to derive their recreational values (Appendix A).

Derivation of an STX RfD

As stated above, NJDEP recreational advisories for cyanotoxins including STX are intended to be protective for children's swimming exposures during cyanoHAB events, which may persist for several months during the swimming season (USEPA, 2019; NJDEP, 2020). The STX RfD derived herein is intended to be protective for exposure on multiple days of swimming during the swimming season, for the more sensitive sub-population of children. Accordingly, laboratory

[1] The most commonly used method of expressing the amount of STX in shellfish implicated in PSP is the mouse bioassay. This assay provides a measure of the total of all STX analogues present within a sample (i.e., this approach can neither qualitatively differentiate among the different STX analogue structures nor provide a quantitative measurement for each individual analogue). Therefore, measurements of STX-group toxins are collective measures of all STX analogues in a sample and have by convention been expressed as STX equivalents (eq) (ESFA 2009; FAO, 2011).

animal and human studies investigating the effects of less than sub-chronic[2] exposure to STX were considered for deriving the short-term RfD.

In addition to studies identified through literature searches, studies reviewed herein include those cited as the basis of recreational guidance values for other states.

Animal toxicology studies

A limited number of studies in laboratory animals exposed to STX were identified. Studies identified primarily involve either acute or subchronic exposure. Studies in which isolated (i.e., pure) STX was administered orally, either by drinking water or diet, are reviewed below as the potential basis for derivation of an STX RfD. Studies in which laboratory animals were exposed to STX along with its analogues are reviewed as supporting data useful for informing results from pure STX exposures. Reviews of available studies of STX using other routes of exposure (e.g., intravenous or intraperitoneal), which are less relevant than oral studies for recreational exposure through incidental ingestion of water, are reviewed elsewhere (Testai et al., 2016; WHO, 2020). Except for neurotoxicity, there is a lack of standard systemic toxicity studies assessing endpoints such as organ weight and histopathology and clinical chemistry. Additionally, the lack of chronic (i.e., > 90 days of exposure), developmental, and reproductive studies, as well as studies focused on genotoxicity and carcinogenicity, identified for this assessment and in recent reviews (Testai et al., 2016; WHO, 2020) gives an indication of the limited extent of the laboratory animal database for STX.

Two oral mouse studies of pure STX, both acute in duration (i.e., a single exposure), were identified (Munday et al. 2013; Finch et al. 2018). Studies by Ramos et al. (2014) and Diehl et al. (2016) reported on the short-term (i.e., repeated dosing up to ~30 days) exposure of rats to drinking water containing cyanobacterial cultures producing STX and its analogues.

Munday et al. (2013)

Female Swiss albino mice received a single oral gavage exposure to STX (> 98% pure). The authors did not explicitly state the doses (i.e., expressed in mass of STX per body weight) to which the mice were exposed nor the number of animals per dose group. After dosing, the mice were observed for neurological effects including grip strength, exploratory behavior, abdominal breathing, and lethargy. Based on lethargy and decreases in grip strength and exploratory behavior, the authors identified a NOAEL of 544 nmol/kg (163 µg/kg)[3].

This study affirms the neurotoxicity of STX. However, a number of factors render this study problematic for RfD development. As noted above, some of the specific doses employed in this study were not reported by the authors. Additionally, the authors did not provide a detailed methodological description of the neurotoxicity tests conducted, the number of animals per dose

[2] The USEPA defines subchronic exposure as occurring for "more than 30 days up to approximately 90 days in typically used laboratory animal species". https://iaspub.epa.gov/sor_internet/registry/termreg/searchandretrieve/glossariesandkeywordlists/search.do?details=&vocabName=IRIS%20Glossary#formTop

[3] All conversions herein from nmol/kg to µg/kg are based on the molecular weight of 299 grams/mole for STX.

group, or quantitative data and the statistical analyses used to detect differences in neurotoxic effects between dose groups.

Finch et al. (2018)

Female Swiss albino mice received a single dietary exposure (via cream cheese) to STX (> 99% purity). The authors did not explicitly state the doses (i.e., expressed in mass of STX per body weight) to which the mice were exposed. Although not explicitly stated, it appeared that there were 3 animals per dose group. After dosing, the mice were continuously monitored for 3 hours for signs of neurotoxicity including any change in posture, respiratory rate, or movement. The authors identified a NOAEL of 1270 nmol/kg (379 µg/kg), although effects were observed in 1 of 3 mice in that dose group. However, 3 of 3 mice showed no effects with exposure to 1140 nmol/kg (341 µg/kg). In the 14 days following exposure, the authors reported that the mice appeared and behaved normally, gained weight, and showed no abnormalities at necropsy.

This study also affirms the neurotoxicity of STX. However, a number of factors render this study problematic for RfD development. As noted above, some of the specific doses employed in this study were not reported by the authors. The authors did not provide a detailed methodological description of the neurotoxicity tests conducted nor the quantitative data and the statistical analyses used to detect differences in neurotoxic effects between dose groups.

Ramos et al. (2014)

Female Wistar rats (5 to 10 per dose group) were orally exposed for 30 days via drinking water contaminated with cyanobacterial (*Cylindrospermopsis raciborskii*) cultures producing STX and its analogues at final concentrations of 3 or 9 µg STX eq/L. Rats in the control group were exposed to drinking water with culture medium but no cyanobacteria. Based on these concentrations, the authors estimated the doses to be 0.24 or 0.72 µg STX eq/day[4]. After the 30 days of exposure, the rats were killed and various subclinical biochemical parameters were assessed in brain (prefrontal cortex, hippocampus) and liver tissues. Specifically, the authors measured the following: concentration of reactive oxygen species (ROS), total antioxidant capacity (ACAP), glutathione (GSH) concentration and glutamate cysteine ligase activity (GCL), glutathione-S-transferase (GST) activity, and lipid peroxidation via the ferrous oxidation-xylenol orange (FOX) assay and the thiobarbituric acid reactive substances (TBARS) assay.

Exposed rats showed no clinical signs of toxicity (not specified by the study authors) and did not have deviations in body weight gain compared to controls. Some changes in biochemical parameters were observed relative to the control group. Although the authors found a decrease in ROS only in the hippocampus of the 3 µg/L group, they also observed a lower ACAP in this group, while ACAP was higher in the cortex of the 9 µg/L group. GCL activity was decreased in the cortex of the 3 µg/L group but increased in the cortex and hippocampus of the 9 µg/L group. GSH levels were increased in the hippocampus and liver of the 3 µg/L group. GST activity was increased in the hippocampus but decreased in the liver of the 9 µg/L group. While lipid peroxidation was increased (FOX assay) in the liver of the both dose groups, no change in lipid peroxidation was observed with the TBARS assay.

[4] Authors reported that each rat drank 0.08 L of contaminated drinking water per day.

This study provides mechanistic information demonstrating the ability of STX and its analogues to affect various parameters associated with oxidative stress in the brains and livers of rats. These changes may precede more serious effects in these tissues such as overt neurotoxicity or liver damage. Nevertheless, the changes in subclinical biochemical parameters assessed in this study are not considered to be adequate to serve as the basis of an RfD (i.e., these endpoints are not specific indicators of an adverse clinical effect or disease). Although this study provided sufficient methodological and statistical details, the use of drinking water contaminated with cyanobacteria raises the possibility that endotoxins produced by the bacteria may confound effects purported to be from STX and its analogues.

Diehl et al. (2016)

Female Wistar rats were orally exposed for 30 days via drinking water contaminated with cyanobacterial (*Cylindrospermopsis raciborskii*) cultures producing STX and its analogues at final concentrations of 3 or 9 µg STX eq/L. Based on these concentrations, the authors estimated the exposure to be 0.24 or 0.72 µg STX eq/day for each rat. The authors checked the *C. raciborskii* cultures for the presence of other cyanobacteria toxins, specifically cylindrospermopsin and microcystin, and found no positive results for their production. A negative control group was exposed to drinking water contaminated with a culture of the cyanobacteria *Aphanothece* sp.[5], which did not produce the toxins listed above. Between 10 and 15 rats were assigned to each dose group. After the 30 days of exposure, the rats were subjected to the following behavioral tests: open field habituation (OFH) task, elevated plus maze anxiety (EPM) task, inhibitory avoidance (IA) task, and Morris water maze (MWM).

Compared to controls (i.e., rats exposed to drinking water with culture medium but no *C. raciborskii*), exposed rats showed no clinical signs of toxicity (not specified by the study authors) and did not have deviations in body weight gain. The following behavioral results were observed relative to the control group. Exposure to STX had no effect on the performance of rats in the OFH and EPM tasks. However, performance was affected in the IA task in rats exposed to 9 µg STX eq/L. Additionally, performance was also affected for certain aspects of the MWM task, such as an increased time to find a hidden platform and time spent within certain quadrants of the test chamber, although only the 9 µg STX eq/L exposure group was tested. Based on the affect in the IA task, a NOAEL of 3 µg STX eq/L is identified, which is converted to approximately 0.8 to 1.1 µg STX eq/kg/day, based on the initial body weight range (210 to 300 g) and drinking water volume (0.08 L/day) of the rats in this study.

Diehl et al. (2016) demonstrates that short-term STX exposure through drinking water contaminated with cyanobacteria can cause memory impairment in rats, an effect not observed or assessed in other studies. In an earlier study by these authors, oxidative stress was reported to occur in the brain of rats exposed to similar conditions (Ramos et al., 2014). This earlier study provides mechanistic support to the observation of memory impairment. However, Diehl et al (2016) is not judged to be adequate for deriving an RfD. Although this study provided sufficient methodological and statistical details, the use of drinking water contaminated with cyanobacteria raises the possibility that endotoxins produced by the bacteria may confound results purported to

[5] Within the context of the Diehl et al. (2016) study, "sp." is referring to the fact that the *Aphanothece* used in this study was a non-toxin producing cyanobacteria that was not taxonomically defined.

be from STX and its analogues. The ability of endotoxin to affect memory in laboratory rodents is reviewed elsewhere (Zakaria et al., 2017; Batista et al., 2019). For some behavioral tests, only one dose group was tested. Additionally, Diehl et al. (2016) do not provide details about how they estimated STX eq in drinking water and do not provide quantitative information for other STX analogues they measured (Vilariño et al., 2018).

Human epidemiology studies

No studies of any duration that investigated the effect of STX-only exposure on human health were identified. However, a number of case reports exist describing PSP in humans, which is caused by STX and its analogues (EFSA, 2009). DSR did not individually assess these case reports as a number of previous assessments have compiled such studies for the derivation of RfDs for STX (Fitzgerald et al., 1999; FAO, 2004; EFSA, 2009; Arnich and Thébault, 2018). As mentioned below, the RfDs from some of these assessments have served as the basis for guidance values used by other US states and some countries.

Four previous assessments were identified that have compiled available case reports for PSP and either identified a POD (Arnich and Thébault, 2018) or actually derived an STX RfD (Fitzgerald et al., 1999; FAO, 2004; EFSA, 2009). These four assessments are summarized below to inform their potential use as the basis for the NJDEP STX RfD. Each of these assessments typically used a similar collection of PSP case reports for deriving an RfD, and the specific case reports considered can be found within each assessment. As PSP results from acute exposure to STX and its analogues, acute RfDs are derived by these assessments. Additionally, as the case reports that informed these RfDs did not differentiate between STX and its analogues causing PSP, the acute RfDs are expressed in µg STX eq/kg. As presented below in the "Discussion" section, a number of uncertainties exist with these case reports.

<u>Fitzgerald et al. (1999)</u>

This assessment, which is published in a peer-reviewed journal, is based on 11 studies reviewing case reports published between the 1950s and 1990s describing PSP in Asia, Europe, North America, and South America. This collection of case reports included 999 exposed individuals (880 with symptoms, 119 without symptoms). Although not completely characterized by Fitzgerald et al. (1999), ages of individuals likely ranged from 2 to >27 years old. Fitzgerald et al. (1999) considers the largest number of individuals compared to the other assessments reviewed herein. However, this sample size is based on the inclusion of a study by Fu et al. (1982), which is not considered by the other assessments but also does not report any human exposure data.

Of these case reports, nine provide human dose information for outcomes (i.e., unaffected, ill, death). However, Fitzgerald et al. (1999) does not report on the methods (e.g., the mouse bioassay assay [MBA]) used to determine the human doses. Table 1 below summarizes outcome and human dose information as reported in Fitzgerald et al. (1999). There is considerable overlap between outcome groups and human doses. For example, the human dose range that caused mortality falls within the dose ranges for unaffected and ill individuals. Such dose ranges demonstrate either a wide range of human susceptibility to PSP, differences in exposure and outcome assessment, or a combination of both.

Table 1. Summary of case report data from Fitzgerald et al. (1999)[a]	
Outcome	Human Dose (µg STX eq) [Human dose in µg STX eq/kg body weight][b]
Unaffected (n = 119)	17 to 36,580 [0.28 to 610]
Ill (n = 828)	13 to 123,457 [0.22 to 2058]
Deaths (n = 52)	456 to 6300 [7.6 to 105]
a = Adapted from Table 1 of Fitzgerald et al. (1999) b = For a rough comparison with results from other European assessments reported herein (e.g., EFSA, 2009), a body weight of 60 kg was assumed for all individuals regardless of age, as Fitzgerald et al. (1999) did not provide a breakdown of the number of individuals in each age group.	

Based on nonfatal illness, Fitzgerald et al. (1999) identified 4 individuals with the lowest reported toxin doses as candidates for the basis of their RfD: an adult (age not specified) exposed to 13 µg toxin, a 2-year old child exposed to 114 µg toxin, a 12-year old male exposed to 124 µg toxin, and a 27-year old female exposed to 124 µg toxin. Fitzgerald et al. (1999) considered the first case (adult exposed to 13 µg toxin) to be an outlier and selected the dose of 124 µg toxin in the adult female as the LOAEL for STX. This selection was based on the fact that when normalizing the total doses (i.e., µg toxin/person) based on age-appropriate body weights (60 kg for an adult) the LOAEL on a body weight basis in the adult female (2.1 µg/kg) was lower than in the 2-year old and 12-year old children.

For UFs, Fitzgerald et al. (1999) applied a factor of 10 to extrapolate from the LOAEL of 2.1 µg/kg identified above to a NOAEL. No UF was applied for human variation as the authors noted that case reports were from several countries, and included both males and females, and adults and children. No additional UFs were applied. The resulting acute RfD is 0.21 µg STX eq/kg.

Using the basis of this LOAEL (i.e., observed health effects with 124 µg of toxin exposure) and recognizing that there were insufficient data to derive a drinking water guideline, Australia's National Health and Medical Research Council (NHMRC, 2011) developed a drinking water health alert value (3 µg/L) for STX (based on 50% of STX exposure coming from drinking water, a daily water consumption rate of 2 L/day, and an uncertainty factor of 10 for use of a LOAEL rather than a NOAEL).

Food and Agriculture Organization of the United Nations (FAO, 2004)

This terse assessment is based on 20 incidents of PSP in Canada between 1970 and 1990 involving about 60 individuals with ages between 3 and 72 years. FAO (2004) does not describe

the methods used to estimate human STX dose, but reports that individuals with mild cases had consumed between 2 and 30 µg/kg while more severe cases consumed > 10 to 300 µg/kg. Based on these data, FAO (2004) identified a LOAEL of 2.0 µg/kg.

For UFs, FAO (2004) applied a factor of 3 to extrapolate from the LOAEL of 2.0 µg/kg to a NOAEL. No UF was applied for human variation as FAO (2004) noted that the cases of PSP involved a spectrum of people in terms of occupation, age, and sex and that mild symptoms were reversible. No other UFs were applied. The resulting acute RfD is 0.7 µg STX eq/kg.

European Food Safety Authority (EFSA, 2009)

This assessment is based on 14 studies reviewing case reports published between the 1940s and 2000s describing PSP in Africa, Europe, North America, and South America. This collection of case reports included over 600 individuals (roughly 574 with symptoms and 80 without symptoms). Although not completely characterized by EFSA (2009), ages of individuals likely ranged from < 6 years old to adults.

EFSA (2009) reported toxin concentrations in shellfish as well as analytical methods and assumptions used to determine human dose. Table 2 below summarizes outcome and human dose information as reported in EFSA (2009). The overlap between outcomes and human doses may be due to the wide range of human susceptibility to PSP, differences in exposure and outcome assessment, or a combination of both.

Table 2. Summary of case report data from EFSA (2009)[a]

Outcome	Human Dose[b] (µg STX eq/kg body weight)
No symptoms	0.3 to 610
Mild symptoms	0.7 to 70
Moderate symptoms	1.5 to 150
Severe symptoms	1.5 to 300
Respiratory arrest/failure	53 to 2058
Death	7 to 225

a = Adapted from Table 16 of EFSA (2009); number of individuals in each outcome category not provided for all studies
b = As reported in EFSA (2009)

Based on these PSP data, EFSA (2009) identified a LOAEL "in the region of 1.5 µg STX equivalents/kg b.w." (i.e., they qualitatively identified the LOAEL). To support this LOAEL, EFSA (2009) stated that "many individuals did not suffer adverse reactions at much higher intakes and therefore it is expected that this LOAEL is very close to the threshold for effects in the most sensitive individuals." This conclusion, however, does not appear to be clearly supported in EFSA (2009), as there is a lack of individual data (e.g., dose and outcome) for the entire study population, including a lack of data on STX levels that caused effects in children versus adults.

For UFs, EFSA (2009) applied a factor of 3 to extrapolate from the LOAEL of 1.5 µg/kg identified above to a NOAEL. No UF was applied for human variation as EFSA noted that "data were from reports of a large number of affected consumers, including the most sensitive individuals." No additional UFs were applied. The resulting acute RfD is 0.5 µg STX eq/kg.

As noted above, five US states (CO, OH, OR, PA, WA) have used this acute RfD and exposure factors, which differed among states, to derive recreational water guideline levels for STX (ranging from 0.8 to 75 µg/L). In addition to these states, the WHO (2020) developed its recreational water guideline value of 30 µg /L for STX based on the LOAEL derived by EFSA (2009).

Arnich and Thébault (2018)

This assessment, which is published in a peer-reviewed journal, developed a quantitative approach (1) to model the dose-response relationship between human exposure to paralytic shellfish toxins (i.e., STX and its analogues) and the severity of PSP symptoms, and (2) to identify a threshold dose for PSP symptoms. In doing so, the authors conducted a systematic review, an investigative process aimed at minimizing bias and maximizing transparency of their assessment. As part of this process, the authors identified all existing published studies on this topic and assessed the quality of each study for use in statistical analysis.

Although Arnich and Thébault (2018) identified 30 studies reviewing case reports of PSP published through February 2018 that reported on 329 exposed individuals, the authors excluded a number of studies due to missing information (e.g., amount of shellfish ingested, temporality between when contaminated shellfish was ingested and collected for analysis). When possible, assumptions were made for missing data (e.g., body weight). Based on this screening step, the authors based subsequent statistical analyses on 191 exposed individuals (149 with symptoms, 42 without symptoms) from 16 studies.

For these analyses, the authors used an ordinal scale (i.e., data were placed into categories of increasing rank) for PSP symptoms based on EFSA (2009):

- 0, no symptoms
- 1, mild symptoms (e.g., dizziness, headache, nausea, numbness, tingling, vomiting)
- 2, moderate symptoms (e.g., incoherent speech, lack of voluntary movement, rapid pulse, shortness of breath)
- 3, severe symptoms (e.g., difficulty swallowing, muscular paralysis, respiratory arrest without death)
- 4, death

In attempting to describe the relationship between STX dose and PSP symptoms, the authors found it necessary to determine whether additional studies should be excluded from dose-response analysis. For example, one study with 7 exposed individuals (5 with symptoms, 2 without symptoms) was excluded from further analysis because toxin exposure for individuals without symptoms was higher than exposure for individuals with symptoms.

Using the 15 remaining studies, Arnich and Thébault (2018) found no clear dose-response relationship between category of symptoms and toxins ingested (in µg STX eq/kg body weight) when displayed graphically on a log10 scale.

With the intent of using the highest quality studies available for dose-response analysis, the authors then assigned a level of confidence (low, medium, or high) to each of the 15 studies. High confidence studies used few assumptions to estimate dose, analyzed toxins in shellfish leftover from the meal consumed by the subject, and reported the amount of shellfish consumed. In contrast, low confidence studies used many assumptions to estimate dose, analyzed other shellfish (i.e., those not consumed by the exposed individual), and were ambiguous about the amount of shellfish consumed. Studies not easily classified into the high or low confidence level were assigned a medium level. Of the 15 studies, the authors assessed that 6 had a low level of confidence, 7 had a medium level, and 2 a high level. Due to small sample size, the authors could not establish a dose-response relationship from a graphical presentation of the data from only the high-level studies. When high and medium level studies were considered, Arnich and Thébault (2018) found that the dose-response results were no better than when using studies with all levels of confidence.

As a further attempt to identify and exclude studies with anomalous data, Arnich and Thébault (2018) conducted a rough sensitivity analysis by determining to what extent the exclusion of a given study affected the R^2 (coefficient of determination) for the entire dataset of all 15 studies. Based on this approach (Figures 6 and 7 in Arnich and Thébault, 2018), the authors found that excluding 2 studies, both of which reported exposure data far from the mean exposure values (for all studies) for some symptom categories, improved the R^2 value from 0.0074 to 0.299. The remaining 13 studies yielded a linear dose-response relationship (p-value < 0.001).

Based on data from the remaining 13 studies of 143 exposed individuals (113 with symptoms, 30 without symptoms), Arnich and Thébault (2018) conducted a dose-response analysis to identify a threshold for PSP symptoms. Although benchmark dose (BMD) modeling is recommended for dose-response analysis by the USEPA (2012), BMD modeling of ordinal data was not available when the Arnich and Thébault (2018) study was conducted. Therefore, the authors developed an approach based on a cumulative link mixed model, which is a standard choice for modeling ordinal data.

To identify the best fitting model, the authors tested whether different fixed effects[6] (e.g., age, dose, sex) and random effects (e.g., publication bias) predictor/explanatory variables were necessary for inclusion in the model. Additionally, the authors explored the use of different link functions (logistic versus probit) for using the actual response data (i.e., the categories of symptoms) from the human case reports to ultimately predict the probability of a given symptom based on STX exposure. Based on this approach and selection of the model with the lowest Akaike Information Criterion (AIC)[7], the authors conducted subsequent analyses using a probit model, with Log10 (dose) and the random effect of publication bias removed.

Using this probit model, Arnich and Thébault (2018) generated prediction curves with 95% confidence intervals. From these curves, the authors identified critical doses (CDs) estimated for

[6] Fixed effect variables are factors assumed to be either constant or to change at a constant rate over time. Random effect variables are assumed to be unpredictable.

[7] Akaike's Information Criterion (AIC) is a statistical measure used to compare how well different nested models (i.e., different combinations of the explanatory variables) predict the response variable. In practice, the model with the lowest AIC is considered the best fit (USEPA, 2012).

a 10%, 5%, and 1% probability of showing symptoms. For each CD, the authors estimated lower and upper critical doses (LCD and UCD) from the 95% lower- or upper-bound of the 95% confidence interval of each CD. This approach is comparable to the identification of the lower- and upper-bound of the BMD (i.e., the BMDL and BMDU) in USEPA's approach to BMD modeling (USEPA, 2012). Table 3 reports the results of these predictions. Based on these predictions, the LCD with a greater than 10% probability of showing any symptom is 0.37 µg STX eq/kg. At this dose, 10% of individuals would have some symptoms of PSP, without consideration of severity of symptoms. The rationale for selecting a 10% risk level, as opposed to a 5% or 1% level, is presented in the "Selection of principal study" section. Additionally, as discussed below, 0.37 µg STX eq/kg can serve as the POD for the derivation of an STX RfD.

Selection of principal study

Both laboratory animal and epidemiology studies that could potentially inform the derivation of a short-term RfD for STX were reviewed. When epidemiology studies that provide appropriate data are available, they are preferable to laboratory animal studies for the derivation of an RfD. Therefore, information on human cases of PSP are considered for deriving the short-term RfD. In addition, the available animal studies were judged not appropriate for the derivation of a short-term STX RfD for reasons mentioned above (e.g., inadequate study design or data reporting, confounding by other bacterial toxins, assessed only sub-clinical endpoints).

The human case reports of PSP from STX exposure have been summarized in four assessments. All of these assessments identified PODs that were in many cases then used to derive acute RfDs for STX (Fitzgerald et al., 1999; FAO, 2004; EFSA, 2009; Arnich and Thébault, 2018). Table 4 summarizes the acute RfDs derived by these assessments. Unlike the other three assessments, Arnich and Thébault (2018) did not derive an RfD from their POD (0.37 µg STX eq/kg). As discussed in more detail below, a POD of 0.37 µg STX eq/kg, based on a 10% risk of any symptom from exposure to STX is judged to be appropriate for use as a POD. This approach is consistent with the USEPA's BMD modeling, in which 10% excess risk (i.e., a 10% response rate over controls or non-exposed individuals) is the default response level (i.e., the benchmark response [BMR]), particularly for data that are not continuous (USEPA, 2012). For the purposes of comparison with the other assessments in Table 4, the POD from Arnich and Thébault (2018) is simply used as the acute RfD. The acute RfDs derived from information in the four

Table 3. Predicted critical doses (in µg STX eq/kg) for each symptom category at different levels of risk			
Level of risk	LCD	CD	UCD
Category of symptoms > 0 (no symptoms)			
10%	0.37	0.88	2.6
5%	0.20	0.47	1.8
1%	0.06	0.14	1.2
Category of symptoms > 1 (mild symptoms)			
10%	1.9	3.7	7.9
5%	0.94	2.0	5.3
1%	0.28	0.60	3.1
Category of symptoms > 2 (moderate symptoms)			
10%	5.2	9.2	17
5%	2.6	4.9	11
1%	0.74	1.5	5.7
Category of symptoms > 3 (severe symptoms)			
10%	82	140	340
5%	43	73	180
1%	13	25	69
Adapted from Table 4 of Arnich and Thébault (2018) with critical doses rounded to two significant digits. CD = critical dose. LCD = lower critical dose. UCD = upper critical dose.			

Table 4. Summary of acute RfDs				
	Fitzgerald et al. (1999)	FAO (2004)	EFSA (2009)	Arnich and Thébault (2018)[a]
Point of Departure (µg STX eq/kg)	2.1 (LOAEL)	2.0 (LOAEL)	1.5 (LOAEL)	0.37 (modeled)[b]
UF_{Animal}	*1*	*1*	*1*	---[c]
UF_{Human}	*1*	*1*	*1*	---
$UF_{Duration}$	*1*	*1*	*1*	---
UF_{LOAEL}	10	3	3	---
$UF_{Database}$	*1*	*1*	*1*	---
UF_{Comp}	10	3	3	---
Acute RfD (µg STX eq/kg)	0.21	0.67	0.5	0.37
a = Arnich and Thébault (2018) did not derive an acute RfD. For comparative purposes, the modeled POD was used as the acute RfD. b = lower confidence level on modeled dose for 10% probability of symptoms c = Dashed lines indicate that Arnich and Thébault (2018) did not apply UFs to their POD. Note: For some assessments in this table, not all UFs (e.g., database) were considered by that assessment. In such cases, DSR applied a 1 (see italics). In no instance did this application change the published acute RfD from that assessment.				

assessments are all within a factor of 3 of each other, with the lowest being 0.21 µg STX eq/kg (Fitzgerald et al., 1999) and the highest 0.67 µg STX eq/kg (FAO, 2004). This is not surprising as many of the same case studies are used in each assessment. Additionally, the composite uncertainty (i.e., the UF_{Comp}) among the assessments is within a factor of 10.

As NJDEP recreational advisories are intended to protect for repeated daily exposures during the duration of a cyanoHAB event (NJDEP, 2020), short-term RfDs are derived in Table 5 from the same four assessments included in Table 4 to account for this exposure duration (i.e., extrapolating from acute to short-term exposure).

None of the acute RfDs shown in Table 4 accounted for deficiencies in the STX database (e.g., lack of systemic, developmental, and reproductive studies). As discussed below, DSR concludes that factors of 3 for human variability (UF_{Human}) and duration ($UF_{Duration}$) as well as a factor of 10 for database deficiencies ($UF_{Database}$) to be appropriate for deriving a short-term RfD for STX. Accordingly, the short-term RfDs in Table 5 are 100-fold lower than their respective acute RfDs in Table 4.

Table 5. Summary of assigned UFs and short-term RfDs derived by DSR from PODs developed by other investigators

	Fitzgerald et al. (1999)	FAO (2004)	EFSA (2009)	Arnich and Thébault (2018)
Point of Departure (µg STX eq/kg)	2.1 (LOAEL)	2.0 (LOAEL)	1.5 (LOAEL)	0.37 (modeled)[a]
UF_{Animal}	1	1	1	1
UF_{Human}	3	3	3	3
$UF_{Duration}$	3	3	3	3
UF_{LOAEL}	10	3	3	1
$UF_{Database}$	10	10	10	10
UF_{Comp}	1000	300	300	100
Short-term RfD (µg STX eq/kg/day)	0.0021	0.0066	0.005	0.0037

a = lower confidence level on modeled dose for 10% probability of symptoms

As with the acute RfDs in Table 4, the short-term RfDs are within a factor of 3 of each other, with the lowest being 0.0021 µg STX eq/kg (Fitzgerald et al., 1999) and the highest 0.0066 µg STX eq/kg (FAO, 2004). The composite uncertainty (i.e., the UF_{Comp}) among the assessments is within a factor of 10.

With the exception of Arnich and Thébault (2018), the other assessments qualitatively (i.e., not through modeling the data) determined the POD (in each case a LOAEL) for deriving an RfD. While yielding the lowest short-term RfD, Fitzgerald et al. (1999) based their POD on a single individual with non-fatal PSP. FAO (2004) identified their POD on the lowest dose causing mild PSP symptoms in the case reports they reviewed. Similarly, EFSA (2009) identified their POD

based on the lowest dose causing moderate symptoms. In contrast, Arnich and Thébault (2018) modeled data from 143 exposed individuals to predict a dose with a 10% chance of causing any PSP symptom. Because of the systematic review approach used to identify and assess relevant data, subsequent quantitative modeling of PSP data, and peer-review, the POD derived by Arnich and Thébault (2018) is deemed most scientifically appropriate for deriving a short-term RfD for STX. Therefore, Arnich and Thébault (2018) is selected as the principal study for the derivation of an STX short-term RfD and recreational guidance value.

Although Arnich and Thébault (2018) did not use USEPA BMD modeling software for determining the POD of 0.37 µg STX eq/kg, their modeling approach is judged to be scientifically appropriate. Overall, the Arnich and Thébault (2018) approach closely resembles the modeling performed by the USEPA BMD modeling software. Arnich and Thébault (2018) selected risk levels (i.e., 10%, 5%, and 1%) for PSP symptoms and then identified doses (CDs) corresponding to those levels of risk with LCDs and UCDs indicating the statistical confidence limits (i.e., 95%) of the CDs. This selection of risk level(s) and identification of doses with confidence limits are analogous to the BMRs, BMDs, and BMDLs and BMDUs employed in BMD modeling recommended by USEPA (2012). With BMD modeling, the BMDL serves as the POD as it accounts for experimental variability and ensures that the BMR is not exceeded (USEPA, 2012). Analogously, the LCD determined in Arnich and Thébault (2018) serves as the POD.

The POD of 0.37 µg STX eq/kg determined in Arnich and Thébault (2018), based on a 10% risk level for any symptom from exposure to STX, is judged to be appropriate, as 10% excess risk (i.e., a 10% response rate over controls or non-exposed individuals) is the default response level (i.e., the BMR) used for BMD modeling, particularly for data that are not continuous (USEPA, 2012). Basing the POD on a lower risk level (e.g., 5% or 1% of showing symptoms) is deemed to be not necessary, as lower risk levels are meant to protect against frank (i.e., more severe) effects. The POD determined in Arnich and Thébault (2018), based on the LCD for a 10% probability of showing any PSP symptom (mild, moderate, severe, death) is 0.37 µg STX eq/kg. This value is far lower than the LCD (1.9 µg/kg) for a 10% probability of even mild symptoms (Table 3). Additionally, a 10% risk level is also judged appropriate because the PSP symptoms (i.e., mild, moderate, and severe) are reversible. Arnich and Thébault (2018, Table 3 therein) estimate that at a dose of 1 µg STX eq/kg (which is over 2.5-fold higher than then POD of 0.37 µg STX eq/kg), the probability of experiencing moderate symptoms, severe symptoms, or death was 1.57%, 0.526%, and 0.002%, respectively. Therefore, the vast majority of individuals exposed to 0.37 µg STX eq/kg could likely experience no or mild symptoms. Individuals who survive PSP for 24 hours have a high probability of a rapid and full recovery (EFSA, 2009).

Selection of uncertainty factors and derivation of the short-term RfD for STX

Based on USEPA guidance (USEPA, 2002), five individual UFs were considered for deriving a short-term RfD for STX. In deriving the short-term RfD, a UF_{Comp} of 100 is applied to the POD of 0.37 µg STX eq/kg from Arnich and Thébault (2018). The specific UFs are as follows:

UF_{Animal} = 1 (i.e., no adjustment is made). The POD is based on human data.

UF_{Human} = 3. The case reports used to inform the POD are based on human data including 143 individuals from both sexes, different life stages (individuals aged 2 to 69 years old), and from various geographical locations. Although this study population includes this diversity, the complete range of human sensitivity to STX may not have been captured thoroughly, particularly for children. The short-term oral RfD and recreational advisory for STX derived by NJDEP is intended to be protective for oral exposure on multiple days of swimming during the swimming season for children, who receive higher exposures via incidental ingestion of water during swimming than adults (see "Exposure factors and derivation of the STX recreational guidance value" section). As reviewed in WHO (2020), children also appear to be more intrinsically sensitive to STX than adults. In Arnich and Thébault (2018), only about 7% of the study population were known to be under 18 years old[8], and the percent of children age 6-11 (the most highly exposed age group during swimming, see below) would have been even lower. In comparison, the percentages of children under 18 years old in the US and NJ populations are 24% and 22%, respectively[9]. This comparison suggests that the Arnich and Thébault (2018) population only partially represents the effects of STX on children.

Aside from children, other individuals may be sensitive to STX. As discussed in the "Mode of Action" section, there are inter-individual differences in isoforms of the VGSCs that bind STX. These isoforms may differ in their distribution, expression, and sensitivity, potentially explaining the higher sensitivity of some individuals to this toxin. Within the Arnich and Thébault (2018) population, 32% of individuals experienced severe effects from STX exposure, while 6% died. While these individuals may have been exposed to higher doses of STX or the doses may not have been accurately determined (see "Discussion" section), it is also possible that these individuals may truly be more sensitive to STX. In support of this possibility, Table 7 of Arnich and Thébault (2018) shows overlap between the category of symptoms experienced and STX dose for the individuals in the study. Specifically, some individuals with severe symptoms or who died were reported to be exposed to a STX dose that caused less severe symptoms in other individuals.

Finally, although this study population does include some sensitive individuals (e.g., children), the total number of individuals is relatively small (n=143) and other potential sensitive populations may be underrepresented.

Because of these considerations discussed above, a factor of 3 is applied to account for human variability.

[8] The percentage of children is based on individual data reported in Appendix A (table A2) of Arnich and Thébault (2018). Individuals with an age less than 18 years old were considered children. For individuals where the age was not available, Arnich and Thébault (2018) considered them to be adults, as the authors assigned these individuals an estimated adult body weight for dose-response analysis. Based on this approach, the Arnich and Thébault (2018) study population was considered to have 10 children and 133 adults.

[9] The percentage of children is based on the number of children aged less than 18 years old compared to the total population for the US in 2010 (https://www.census.gov/prod/cen2010/briefs/c2010br-03.pdf) and as estimated in NJ in 2019 (https://www.nj.gov/labor/lpa/dmograph/est/nj_agesex2019.xlsx).

$UF_{Duration}$ = 3. The POD is based on PSP in humans following a single (i.e., acute) exposure to STX-contaminated shellfish during a meal. A single exposure is shorter than the intended exposure scenario for the STX recreational value, which is multiple days of swimming during the swimming season. Therefore, in deriving the short-term RfD, the POD based on acute information needs to be adjusted downward to account for a longer period of exposure, in particular to account for the accumulation of STX in the body, for which a human half-life of 10 to 20.4 hours has been reported (Gessner et al., 1997; Wharton et al., 2017). While a swimming season may last many months, it is unlikely that an individual would swim every day during that season. Swimming may more likely occur on consecutive days on a weekly basis, which is consistent with a short-term exposure of between 24 hours and 30 days. Therefore, a factor of 3 is applied to account for duration of exposure.

UF_{LOAEL} = 1. The POD derived from the LCD identified by Arnich and Thébault (2018) (a 10% probability of showing any PSP symptom) is analogous to a BMDL identified through USEPA's BMD modeling. Based on USEPA practice, a factor of 1 is applied to the UF_{LOAEL} when a BMDL is identified and used as the POD. This is based on the assumption that the BMR selected for a critical effect represents a minimal, biologically significant change (USEPA, 2018). Accordingly, a factor of 1 is judged appropriate.

$UF_{Database}$ = 10. The lack of developmental, reproductive, and immune studies identified for this assessment and in recent reviews (Testai et al., 2016; WHO, 2020) gives an indication of the limited extent of the mammalian toxicological database for STX. Therefore, a factor of 10 is judged appropriate to account for the lack of these types of studies.

Short-term RfD = POD / UF_{Comp} = (0.37 µg STX eq/kg/day) / 100 = 0.0037 µg/kg/day.

Although based on a POD expressed in µg STX eq/kg/day due to the likely presence of multiple STX analogues present in the underlying PSP data, the resulting short-term RfD is simply expressed hereafter in µg/kg/day. In practice, this short-term RfD assumes that STX is the most prevalent and toxic of the STX analogues present in a sample (e.g., a surface water sample).

Exposure factors and derivation of the STX recreational guidance value

Recreational guidance values for cyanotoxins, such as STX, are based on exposure through incidental ingestion during swimming. Exposure though incidental ingestion is higher in children than in adults. Factors relevant to incidental ingestion are the incidental ingestion rate (L/h), daily exposure duration (h/day), and body weight (kg). The exposure factors used by NJDEP (2018) and USEPA (2019) are discussed below.

The incidental ingestion rate of 0.12 L/h previously used by NJDEP (2018) in recreational advisories for other cyanotoxins was based on Dufour et al. (2006), which is cited in the USEPA (2011) Exposure Factors Handbook. In this study, the upper percentile (97th percentile) ingestion rate in children less than 18 years old was 0.09 L for a 45-minute swimming event, equivalent to 0.12 L/h. The duration of swimming each day was assumed to be 1 hour by

NJDEP, but this assumption was not based on empirical data. Based on this incidental ingestion rate and swimming duration, daily incidental ingestion during swimming was assumed to be 0.12 L/day.

USEPA (2019) provided additional data on incidental ingestion of children during swimming subsequent to the development of the NJDEP (2018) recreational guidance values for other cyanotoxins. USEPA (2019) developed distributions of incidental ingestion rates (L/h) for different age groups (6-10 years, 11-17 years, 18 and over) based on data from seven studies collected and analyzed by Dufour et al. (2017). This dataset includes 10 times more participants than Dufour et al. (2006), the study cited in the USEPA (2011) Exposures Factors Handbook that was used by NJDEP (2018). Incidental ingestion rates were highest for children age 6-10 years.

Duration of exposure was estimated from data in the USEPA (2011) Exposure Factors Handbook. The data show that children age 5-11 spend more time in the water than younger children, older children, or adults. These data are depicted in a graph (Figure 4-4) in USEPA (2019), where mean, median, and 90th percentiles for daily durations for age 5-11 (who swam at home in the outdoor pool or spa) are about 2.75 h, 2.35 h, and 5 h, respectively.

The distribution of daily incidental ingestion (L/day) was developed by USEPA using Monte Carlo simulations that combined the distributions for incidental ingestion rate (L/h) and duration of exposure (h/day). Daily incidental ingestion (L/day) was higher for age 6-10 than in older age groups. The daily incidental ingestion rate used by USEPA (2019) is 0.21 L/day, which is the 90th percentile of the combined distribution for age 6-10.

DSR has reviewed the basis of the USEPA (2019) exposure assumptions and has concluded that they are more technically sound than the assumptions used by NJDEP (2018). A major difference between the 0.21 L/day ingestion rate from USEPA (2019) and the 0.12 L/day rate from NJDEP (2018) is that the NJDEP (2018) exposure duration of 1 hour was an assumption that was not based on empirical data. The USEPA (2019) daily incidental ingestion rate of 0.21 L/day is the overall 90th percentile ingestion rate for children 6-11 years of age, based on the distributions of both hourly incidental ingestion rate and daily swimming exposure durations. Based on these data, the NJDEP (2018) assumption of a 1-hour exposure duration does not sufficiently represent the daily duration of swimming for children in this age group. Therefore, the NJDEP recreational guidance level for STX is based on a daily incidental ingestion rate of 0.21 L/day.

The equation for deriving the STX recreational guidance value is given as:

Recreational guidance value (µg/L, ppb) = (RfD x BW) ÷ I

Where:
RfD = the Reference Dose for STX (0.0037 µg/kg/day)
BW = the assumed body weight of a child (31.8 kg; based on a mean body weight of children 6 to < 11 years old from USEPA [2011])
I = the daily incidental ingestion rate of swimming water (0.21 L/day)

Based on the equation above, the proposed recreational guidance value for STX is 0.6 µg/L (rounded from 0.56 µg/L).

Discussion

There are numerous uncertainties related to the STX short-term RfD used to derive the recreational guidance value. The literature relevant to the derivation of a short-term RfD for STX is limited, particularly for studies in laboratory animals. In contrast, a number of case reports for human PSP exist and were ultimately used as the basis for the STX short-term RfD. However, as discussed below, a number of uncertainties exist with these case reports.

As discussed elsewhere (FAO, 2004; EFSA, 2009; FAO, 2011; Arnich and Thébault, 2018; WHO, 2020), uncertainties are associated with the available case reports for PSP. Perhaps the biggest concern with these reports is determining the dose of STX that caused PSP symptoms. For example, in determining the concentrations of STX and its analogues present in the implicated food, some reports sampled leftover shellfish consumed at the meal prior to the onset of symptoms, whereas other reports sampled uncooked shellfish from the same batch that was consumed or obtained from the same source (harvesting area, store, restaurant). Other reports may have sampled shellfish harvested on a different day. Compounding the issue of shellfish sampling is accurate determination of the amounts of shellfish consumed and whether cooking affected toxin levels. Additional contributions to the uncertainties of these studies include the body weight of the affected individual (e.g., measured or often assumed to be 60 kg by European assessments), the aptitude of medical staff diagnosing PSP, and the variation in susceptibility within the human population.

Further uncertainties arise from the analytic approach used to determine the amount of STX and its analogues in shellfish. Although chemical approaches exist for measuring the levels of STX and its analogues in shellfish (e.g., high-pressure liquid chromatography coupled with fluorescent detection; reviewed in FAO, 2011), the majority of case reports of PSP relied on the mouse bioassay (MBA) for determining the concentration of toxin present in the food. Historically, the MBA has been the primary method for detecting the presence of STX and its analogues in shellfish around the world, including in New Jersey (FDA, 2012; NJDEP, 2016). In short, this assay involves extracting toxins from a homogenate of the suspected shellfish, exposing mice to the extract by intraperitoneal injection, and monitoring the time it takes for the mice to die. Extracts can be diluted so that death occurs within 5 to 7 minutes, and the amount of dilution needed provides a quantitative metric (i.e., mouse units) that can be converted to µg of STX. As the MBA cannot distinguish between the STX analogues in a sample, the result of the assay is expressed as STX eq (EFSA, 2009; FAO, 2011). Inter-laboratory variability with the MBA adds to the uncertainty with assessing human STX exposure from PSP, as animal characteristics (e.g., strain, sex, general health) and toxin extraction protocols can differ between laboratories (EFSA, 2009). The conversion of mouse units to µg of STX eq is another source of uncertainty.

An important limitation to the use of the human PSP data, not only for Arnich and Thébault (2018) but also the other assessments relying on these data (Fitzgerald et al., 1999; FAO, 2004;

EFSA, 2009), is publication bias. Specifically, the case reports used to inform these assessments were primarily of exposed individuals with symptoms of PSP (i.e., sample selection was biased because it did not follow a randomized sampling design). Exposed individuals without symptoms of PSP are likely underrepresented in the dataset, as only symptomatic individuals are likely to seek medical attention and be included in case reports. Arnich and Thébault (2018) address this limitation by stating "Data on exposure of individual who ate some shellfish but had no symptoms are also very important, in order to better model the dose-response relationship at low doses and get a more accurate estimate of the dose without symptoms. Even if low doses are included in our dataset (Figure 2) from different outbreaks, there is a publication bias on no (sic) symptomatic individuals, and it is possible that our dose-response could over-estimate the risk." Additionally, there is the potential underrepresentation of individuals with mild to moderate symptoms who were exposed to lower levels of STX. Had such individuals been included in the PSP dataset (i.e., they sought medical attention due to their symptoms resulting in a case report), their information (STX dose and symptom category) could have helped inform the lower portion of the dose-response curve. Nevertheless, given these multiple uncertainties, the short-term RfD derived here is intended to be protective and is probably highly conservative (i.e., protective) for the most likely exposures.

Notwithstanding these uncertainties and limitations in the human PSP data, a short-term RfD for STX based on the human data, specifically as analyzed by Arnich and Thébault (2018), is supported by the toxicology data in laboratory animals with acute STX-only exposure. Specifically, studies by Munday et al. (2013) and Finch et al. (2018) exposed female mice to relatively pure STX and reported neurotoxicity. While epidemiological data are preferred over animal data for deriving toxicity values, short-term RfDs could be derived for comparative purposes using these two animal studies. As summarized in Appendix B, short-term RfDs derived from acute STX-only animal studies range from 0.054 µg/kg/day (Munday et al., 2013) to 0.13 µg/kg/day (Finch et al., 2018). These short-term RfDs based on rodent data are more than an order of magnitude higher than the short-term RfD of 0.0037 µg/kg/day based on the analysis of human PSP data by Arnich and Thébault (2018).

It should be noted that multiple analogues of STX are produced by cyanobacteria, and that the analytical assay used by the Bureau of Freshwater and Biological Monitoring measures the total concentration of multiple STX analogues (i.e., it does not measure individual analogues). The short-term RfD is based on the toxicity of STX, as it is considered more prevalent and toxic than most of its analogues. The toxicological database for the STX analogues is insufficient to develop an RfD for any of them. Therefore, it is recommended that the guidance value based on STX cover the whole spectrum on STX analogues present in a given sample.

Comparison with other state recreational guidance value

Five US states (CO, OH, OR, PA, WA) are reported to have recreational water guideline levels for STX (USEPA, 2019). Although all are based on symptoms of PSP from the same principal study (EFSA, 2009), these recreational values range from 0.8 to 75 µg/L (Appendix A). This range in values reflects their intended application to acute (OH, OR, PA, WA) versus short-term exposures (CO), and use of different UFs and exposure factors among these states. OH EPA

(2016) has the lowest recreational value of 0.8 µg/L, and this value is also used by PA DEP (2017). Table 6 compares the basis of the NJDEP and OH EPA (2016) recreational guidance values.

Table 6. Comparison of the basis of the NJDEP and OH EPA recreational guidance values for STX

	NJDEP (2021)	OH EPA (2016)
Critical effect (Principal study)	Symptoms of PSP (Arnich and Thébault, 2018)	Symptoms of PSP (EFSA, 2009)
Point of departure (µg STX eq/kg)	0.37 (modeled)	0.5 (NOAEL)[a]
UF_{Animal}	1	1
UF_{Human}	3	10
$UF_{Duration}$	3	1
UF_{LOAEL}	1	1
$UF_{Database}$	10	10
UF_{Comp}	100	100
RfD (µg/kg/day)	0.0037 Short-term	0.005 Acute
Body weight (kg)	31.8	15
Daily incidental ingestion of swimming water (L/day)	0.21	0.1
Incidental ingestion rate (L/kg/day)	0.006 L/kg/day	0.006 L/kg/day
Recreational value (µg/L)	0.6	0.8

a = The NOAEL of 0.5 µg STX eq/kg was derived by EFSA (2009) based on a LOAEL of 1.5 µg STX eq/kg and the application of a factor of 3.

Although based on an acute RfD, the OH EPA (2016) value is virtually identical to the NJDEP value. However, the basis of the OH EPA value (i.e., EFSA, 2009) is judged to be not as scientifically robust as the basis for the NJDEP value (i.e., Arnich and Thébault, 2018). In short, EFSA (2009) identified a LOAEL "in the region of 1.5 µg STX equivalents/kg b.w." (i.e., the LOAEL was identified qualitatively), which EFSA (2009) then converted to a NOAEL that was used as the POD for the OH EPA value. In contrast, the POD from Arnich and Thébault (2018) is based on a systematic review approach to identify and assess data, statistical modeling, and it underwent peer review.

A notable difference between the OH EPA (2016) and NJDEP recreational values is the application of a factor of 10 to account for human variability. OH EPA applied a full factor of 10, whereas NJDEP applied a partial factor of 3 as the study population in Arnich and Thébault (2018) partially informed the spectrum of human variability by including individuals of both sexes, different life stages (individuals aged 2 to 69 years old), and from various geographical locations. Unlike OH EPA (2016) and NJDEP, other states using EFSA (2009) as the basis for their STX recreational values (WA DOH, 2011; OHA, 2019) applied a factor of 1 for human

variability. Additionally, EFSA (2009) did not apply a factor stating that "No additional factor for variation among humans was deemed necessary because the data covered a large number of affected consumers, including sensitive individuals."

Aside from differences in UFs, derivation of the OH EPA (2016) and NJDEP recreational values differ in terms of exposure factors, specifically body weight of children and incidental water ingestion rate. While these exposure factors are numerically different, the difference between the two states is negated as the ratio between the daily ingestion rate to body weight, which is the incidental ingestion rate (L/kg/day), is virtually identical for OH EPA (2016) and NJDEP.

The only other state to derive an STX recreational value based on a short-term RfD was CO (CDPHE, ND; Appendix A). The CDPHE derived a value of 4 µg/L based on EFSA (2009), which is over 6 times higher than the NJDEP recreational value. The higher values developed by OR (8 µg/L) and WA (75 µg/L) are for acute exposure.

Conclusion

Based on the modeling of human PSP data, a short-term RfD of 0.0037 µg/kg/day for STX was derived. Using the assumed body weight of a child (31.8 kg) and the daily incidental ingestion rate of swimming water (0.21 L/day) from the USEPA (2019), an STX recreational guidance value of 0.6 µg/L was derived and is recommended for use during New Jersey cyanoHAB events.

Acknowledgments

This assessment was reviewed by members of the Division of Science and Research including Drs. Josephine Bonventre, Gary Buchanan, Mingzhu Fang, and Gloria Post. Dr. Lori Lester provided statistical consultation. The constructive comments of three external peer-reviewers are acknowledged.

References

Arnich N, Thébault A. 2018. Dose-Response Modelling of Paralytic Shellfish Poisoning (PSP) in Humans. Toxins. 10: E141.

Batista CRA, Gomes GF, Candelario-Jalil E, Fiebich BL, de Oliveira ACP. 2019. Lipopolysaccharide-induced neuroinflammation as a bridge to understand neurodegeneration. Int J Mol Sci. 20:2293-2323.

CDPHE. N.D. Colorado Department of Public Health and Environment. Algae bloom risk-management toolkit. For recreational waters. https://drive.google.com/file/d/0B0tmPQ67k3NVczRwQkc3Q2dOXzA/view

Diehl F, Ramos PB, Dos Santos JM, Barros DM, Yunes JS. 2016. Behavioral alterations induced by repeated saxitoxin exposure in drinking water. J Venom Anim Toxins Incl Trop Dis. 22:18.

Donovan CJ, Ku JC, Quilliam MA, Gill TA. 2008. Bacterial degradation of paralytic shellfish toxins. Toxicon. 52:91-100.

Dufour AP, Evans O, Behymer TD, Cantú R. 2006. Water ingestion during swimming activities in a pool: a pilot study. J Water Health 4:425-30.

Dufour AP, Behymer TD, Cantú R, Magnuson M, and Wymer LJ. 2017. Ingestion of swimming pool water by recreational swimmers. Journal of Water and Health, 15(3), 429-437. (Cited in USEPA, 2019).

EFSA. 2009. European Food Safety Authority. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Marine Biotoxins in Shellfish – Saxitoxin Group. The EFSA Journal. 1019:1-76. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2009.1019

FAO. 2004. Report of the Joint FAO/IOC/WHO ad hoc Expert. Consultation on Biotoxins in Bivalve Molluscs. Advance pre-publication copy. https://www.who.int/foodsafety/publications/chem/biotoxin_report_en.pdf

FAO. 2011. Food and Agriculture Organization of the United Nations. Assessment and management of biotoxin risks in bivalve molluscs. http://www.fao.org/3/i2356e/i2356e.pdf

FDA. 2012. United States Food and Drug Administration. Bad Bug Book. Handbook of Foodborne Pathogenic Microorganisms and Natural Toxins. https://www.fda.gov/media/83271/download

Finch SC, Boundy MJ, Harwood DT. 2018. The acute toxicity of tetrodotoxin and tetrodotoxin-saxitoxin mixtures to mice by various routes of administration. Toxins.10.E423.

Fitzgerald DJ, Cunliffe DA, Burch MD. 1999. Development of health alerts for cyanobacteria and related toxins in drinking water in South Australia. Environmental Toxicology. 14:203-207.

Fu XC. 1982. Food poisoning caused by eating snails. Zhonghua Yu Fang Yi Xue Za Zhi [Chinese journal of preventive medicine]. 16:140-3.

Gessner BD, Bell P, Doucette GJ, Moczydlowski E, Poli MA, Van Dolah F, Hall S. 1997. Hypertension and identification of toxin in human urine and serum following a cluster of mussel-associated paralytic shellfish poisoning outbreaks. Toxicon. 35:711-722.

Lima-Filho CM, Nogaroli L, Hedin-Pereira C, Azevedo SMFO, Soares RM. 2020. Effects of saxitoxins exposure on oligodendrocyte development in mouse neonates. Toxicon. 188:89-94.

Munday R, Thomas K, Gibbs R, Murphy C, Quilliam MA. 2013. Acute toxicities of saxitoxin, neosaxitoxin, decarbamoyl saxitoxin and gonyautoxins 1&4 and 2&3 to mice by various routes of administration. Toxicon. 76:77-83.

NHMRC. 2011. National Health and Medical Research Council. National Water Quality Management Strategy. Australian Drinking Water Guidelines 6. https://www.nhmrc.gov.au/file/14288/download?token=I0abH9A_

NJDEP. 2016. New Jersey Department of Environmental Protection. State of New Jersey Contingency Plan for the Control of Shellfish Potentially Contaminated by Marine Biotoxin. https://www.nj.gov/dep/bmw/docs/BiotoxinContingency2016.pdf

NJDEP. 2018. New Jersey Department of Environmental Protection. Cyanobacterial Harmful Algal Bloom (HABs) Freshwater Recreational Response Strategy. Revision 1.0 June 2018. https://www.state.nj.us/dep/wms/bfbm/download/NJHABResponseStrategy2018.pdf

NJDEP. 2020. 2020 Cyanobacterial Harmful Algal Bloom (HAB) Freshwater Recreational Response Strategy. June 2020. https://www.nj.gov/dep/hab/download/NJHABResponseStrategy.pdf

OH EPA. 2016. Ohio Environmental Protection Agency. Harmful Algal Bloom Response Strategy for Recreational Waters. https://epa.ohio.gov/portals/35/hab/HABResponseStrategy.pdf

OHA. 2019. Oregon Health Authority. Oregon Harmful Algae Bloom Surveillance (HABS) Program. Recreational Use Public Health Advisory Guidelines Cyanobacterial Blooms in Freshwater Bodies. https://www.oregon.gov/oha/PH/HEALTHYENVIRONMENTS/RECREATION/HARMFULALGAEBLOOMS/Documents/2019%20Advisory%20Guidelines%20for%20Harmful%20Cyanobacterial%20Blooms%20in%20Recreational%20Waters.pdf

PA DEP. 2017. Pennsylvania Department of Protection. Lake Erie Harmful Algal Bloom Monitoring and Response Strategy https://seagrant.psu.edu/sites/default/files/PA%20Lake%20Erie%20HAB%20Response%20Strategy%207-24-2017_0.pdf

O'Neill K, Musgrave IF, Humpage A. 2016. Low dose extended exposure to saxitoxin and its potential neurodevelopmental effects: A review. Environ Toxicol Pharmacol. 48:7-16.

Ramos PB, Diehl F, Dos Santos JM, Monserrat JM, Yunes JS. 2014. Oxidative stress in rats induced by consumption of saxitoxin contaminated drink water. Harmful Algae. 37:68-74.

Smith EA, Grant F, Ferguson CM, Gallacher S. 2001. Biotransformations of paralytic shellfish toxins by bacteria isolated from bivalve molluscs. Appl Environ Microbiol. 67:2345-53.

Testai E, Scardala S, Vichi S, Buratti FM, Funari E. 2016. Risk to human health associated with the environmental occurrence of cyanobacterial neurotoxic alkaloids anatoxins and saxitoxins. Crit Rev Toxicol. 46:385-419.

USEPA. 2002. United State Environmental Protection Agency. A Review of the Reference Dose and Reference Concentration Processes. https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf

USEPA. 2009. United State Environmental Protection Agency. 2009 Edition of the Drinking Water Standards and Health Advisories. https://nepis.epa.gov/Exe/ZyPDF.cgi/P1005P2B.PDF?Dockey=P1005P2B.PDF

USEPA. 2011. United State Environmental Protection Agency. Exposure Factors Handbook 2011 Edition (Final). http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=522996

USEPA. 2012. United State Environmental Protection Agency. Benchmark Dose Technical Guidance. https://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf

USEPA. 2016. United States Environmental Protection Agency. Draft Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. https://www.epa.gov/sites/production/files/2016-12/documents/draft-hh-rec-ambient-water-swimming-document.pdf

USEPA. 2018. United States Environmental Protection Agency. Integrated Risk Information System. Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0313tr.pdf

USEPA. 2019. United States Environmental Protection Agency. Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. https://www.epa.gov/sites/production/files/2019-05/documents/hh-rec-criteria-habs-document-2019.pdf

Vilariño N, Louzao MC, Abal P, Cagide E, Carrera C, Vieytes MR, Botana LM. 2018. Human Poisoning from Marine Toxins: Unknowns for Optimal Consumer Protection. Toxins. 10ii: E324.

WA DOH. 2011. Washington State Department of Health. Washington State Provisional Recreational Guidance for Cylindrospermopsin and Saxitoxin. https://www.doh.wa.gov/Portals/1/Documents/4400/332-118-CylindroSax%20Report.pdf

Wharton RE, Feyereisen MC, Gonzalez AL, Abbott NL, Hamelin EI, Johnson RC. 2017. Quantification of saxitoxin in human blood by ELISA. Toxicon. 133:110-115.

WHO. 2003. Guidelines for safe recreational water environments. Volume 1. Coastal and fresh waters. https://apps.who.int/iris/bitstream/handle/10665/42591/9241545801.pdf;jsessionid=4CFA13A82BB554A640A530118D60F8B8?sequence=1

WHO. 2020. Cyanobacterial toxins: Saxitoxins. Background document for development of WHO Guidelines for Drinking-water Quality and Guidelines for Safe Recreational Water Environments. https://apps.who.int/iris/bitstream/handle/10665/338069/WHO-HEP-ECH-WSH-2020.8-eng.pdf?sequence=1&isAllowed=y

Zakaria R, Wan Yaacob WM, Othman Z, Long I, Ahmad AH, Al-Rahbi B. 2017. Lipopolysaccharide-induced memory impairment in rats: a model of Alzheimer's disease. Physiol Res. 66:553-565.

Zepeda RJ, Candiracci M, Lobos N, Lux S, Miranda HF. 2014. Chronic toxicity study of neosaxitoxin in rats. Mar Drugs. 12:5055-5071.

CHRIS CHRISTIE
Governor

KIM GUADAGNO
Lt. Governor

State of New Jersey

BOB MARTIN
Commissioner

Department of Environmental Protection
Division of Science, Research and Environmental Health
Mail Code 428-01 P.O. Box 420
Trenton, NJ 08625-0420
(609) 984-6070
Fax (609) 292-7340

TO: Patricia Gardner, Director, Division of Water Supply and Geoscience

THROUGH: Gary A. Buchanan, Ph.D., Director
Alan Stern, Dr.P.H., Chief, Bureau of Risk Analysis

FROM: Gloria Post, Ph.D., Research Scientist

SUBJECT: Updated review of USEPA Drinking Water Health Advisories for cyanobacterial toxins and recommendations for NJDEP drinking water guidance

DATE: September 1, 2017

Summary

In June 2015, USEPA issued Drinking Water Health Advisories for two cyanobacterial toxins, microcystin and cylindrospermopsin. USEPA also reviewed information on a third cyanobacterial toxin, anatoxin-a, and concluded that there was insufficient information to develop a Drinking Water Health Advisory for it.

In July 2016, the NJDEP Division of Water Supply and Geoscience requested that DSREH review the basis for the USEPA (2015) Drinking Water Health Advisories for cyanobacterial toxins and make recommendations as to guidance for these toxins in drinking water.

This memo is an update of the earlier October 11, 2016 memo on this topic. This updated memo reflects changes in the earlier draft Reference Doses (RfDs) developed by DSREH for these three toxins. These draft Reference Doses have now been peer reviewed by external experts, and the RfDs for microcystin and anatoxin-a have been revised based on the peer reviewers' comments. The detailed basis for the revised RfDs is presented in the final *NJDEP Recommended NJ Action Level and Health Advisory*

Guidelines for Recreational Exposure to Microcystin-LR, Cylindrospermopsin, and Anatoxin-a, dated August 2017 and posted at http://www.state.nj.us/dep/wms/bfbm/NJHABResponseStrategy.pdf.

Please note that these RfDs were developed by DSREH for use in recreational exposure guidance. As such, they are intended to address **short-term exposures** that might occur for several hours a day, over a limited period, during the outdoor swimming season, and during a harmful algal bloom and are **not intended to address chronic exposure**. With these constraints, these RfDs are **equally applicable to short-term drinking water exposure**, such as the 10-day exposure period envisioned in the USEPA Drinking Water Health Advisories for these toxins.

In summary, DSREH recommends a microcystins guidance value of 0.07 µg/L for infants and children up to 6 years of age, while the USEPA Health Advisory of microcystins for infants and children up to 6 years of age is 0.3 µg/L. This is the guidance value that is likely to be most frequently applied by NJDEP when harmful algal blooms occur in drinking water. The guidance value for microcystins is likely to be the most frequently applied of the three cyanotoxin guidance values. When these guidance values are applied, the guidance value for infants/young children is likely to be applied, rather than the less stringent guidance value for older individuals. The DSREH and USEPA guidance values for cylindrospermopsin for infants and children up to 6 years of age are 0.2 µg/L and 0.7 µg/L, respectively. The DSREH guidance value for anatoxin-a for infants and children up to 6 years of age is 0.7 µg/L, and, as above, USEPA did not develop guidance for this toxin.

Development of drinking water guidance values by DSREH and comparison to USEPA Health Advisories

Health-based drinking water concentrations such as Health Advisories are developed using exposure assumptions and toxicity factors. Both the exposure assumptions and the toxicity factors relevant to development of guidance for these cyanobacterial toxins are reviewed below.

Exposure assumptions:

Exposure duration

USEPA Health Advisories for cyanobacterial toxins are intended to be protective for short-term (10 day) exposure. This exposure duration is based on human exposure scenarios for cyanobacterial blooms in drinking water. **DSREH agrees with the exposure duration selected by USEPA.**

Daily drinking water ingestion rates

USEPA derived separate advisories for 1) bottle fed infants and children up to 6 years of age, and 2) children 6 years of age and older and adults.

The advisories for infants and children up to 6 years old are based on 90th percentile water ingestion average rates for infants from 0-12 months old, 0.15 L/kg/day. This is based on the time-weighted average of the three separate age periods within 0-12 months (0-3 months – 0.23 L/kg/day; 3-6 months – 0.148 L/kg/day; 6-12 months – 0.112 L/kg/day). Within the age group (0-6 years old) to which this advisory is applied, the 90th percentile ingestion rate is somewhat higher than 0.15 L/kg/day in infants 0-3 months old (0.23 L/kg/day).

USEPA recognizes that if advisories had been calculated based on the higher ingestion rate of infants 0-3 months old (0.23 L/kg/day), they would be about 40% lower than the finalized Health Advisories which are based on the time-weighted average for age 0-12 months (0.15 L/kg/day). USEPA states that infants 0-3 months of age are not at disproportionate risk from exposure at a Health Advisory that is higher than the one that would be calculated based on their higher intake because a "safety factor of 30 is built into this calculation to account for human variability and deficiencies in the database." **DSREH agrees with USEPA's conclusion and with its choice of the ingestion rate of 0.15 L/kg/day, based on the time-weighted average for 0-12 months, for developing the Health Advisory for infants and children up to age 6 years.**

The advisories for children 6 years of age and older and adults is based on the 90th percentile water ingestion rate for adults 21 years of age and older (0.034 L/kg/day). USEPA concludes that this exposure assumption is protective of these age groups because the ingestion rate (L/kg/day) for children 6-11 years of age (0.035 L/kg/day) is almost the same as for adults age 21 years and older, and the ingestion rate for ages 11-21 years of age (0.023-0.026 L/kg/day) is lower than for younger children and adults. **DSREH agrees with USEPA's conclusions and with its choice of ingestion rate for children 6 years of age and older and adults.**

Toxicity factors

DSREH developed oral Reference Doses (RfDs) for microcystin-LR, cylindrospermopsin, and anatoxin-a as part of the development of draft recreational exposure guidance for these toxins. **These oral RfDs are also applicable to short-term drinking water exposure but are not intended to address chronic exposure. DSREH recommends that they be used as the basis for New Jersey drinking water guidance for these toxins.**

As mentioned above, the detailed basis for the RfDs developed by DSREH is found in the *NJDEP Recommended NJ Action Level and Health Advisory Guidelines for Recreational Exposure to Microcystin-LR, Cylindrospermopsin, and Anatoxin-a* dated August 2017 and posted at http://www.state.nj.us/dep/wms/bfbm/NJHABResponseStrategy.pdf. This document also includes a detailed comparison of the basis for the DSREH Reference Doses with the Reference Doses from the USEPA (2015) Drinking Water Health Advisories. (These USEPA Reference Doses are also presented in the USEPA (2016) *Draft Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin*.)

Microcystin

The RfDs developed by USEPA and DSREH for microcystins are both based on studies of microcystin-LR in mice.

The basis for the RfD developed by USEPA is liver toxicity at the Lowest Observed Adverse Effect Level (LOAEL) of 50 µg/kg/day in a 28 day mouse study (Heinze et al., 1999). A total uncertainty factor (UF) of 1000 was applied to derive an RfD of **0.05 µg/kg/day**.

The basis for the RfD developed by DSREH is based on a minimal LOAEL of 40 µg/kg/day in a 13 week mouse study (Fawell et al., 1994). At this dose, body weight was significantly decreased, and there were also other effects (changes in total blood protein, albumin, and chronic liver inflammation) that were not statistically significant at this dose but were significant at higher doses. A total uncertainty factor (UF) of 3000 was applied to derive an RfD of **0.01 µg/kg/day**.

The LOAELs used as the basis for the USEPA and DSREH RfDs are similar (USEPA - 50 µg/kg/day; DSREH - 40 µg/kg/day). However, USEPA used a total uncertainty factor (UF) of 1000, while DSREH used a total UF of 3000. This difference results from USEPA's use of a UF for database deficiencies of of 3, while DSREH used a value of 10. The reason for the application of the higher uncertainty factor by DSREH is explained in detail in the NJDEP document mentioned above.

Cylindrospermopsin
Both USEPA and DSREH developed RfDs for cylindrospermopsin based on the No Observed Adverse Effect Level (NOAEL) of 30 µg/kg/day from an 11-week mouse study (Humpage and Falconer, 2003). At higher doses, increased relative kidney weight occurred.

USEPA used a total uncertainty factor of 300, including an uncertainty factor of 3 for deficiencies in the database, to derive an RfD of **0.1 µg/kg/day**.

DSREH used a total uncertainty factor of 1000, including an uncertainty factor of 10 for deficiencies in the database, to derive an RfD of **0.03 µg/kg/day**. The reason for the application of the higher uncertainty factor by DSREH is explained in detail in the NJDEP document mentioned above.

Anatoxin-a
USEPA concluded that there was insufficient information to derive an RfD for anatoxin-a.

DSREH derived an RfD based on the NOAEL of 98 µg/kg/day from a 4 week mouse study (Fawell and James, 1994; Fawell et al., 1999). A total uncertainty factor of 1000 was applied to derive an RfD of **0.1 µg/kg/day**.

Drinking water guidance values recommended by DSREH and comparison to USEPA Health Advisories
The short term drinking water guidance values for infants/young children and children over 6 years/adults based on the DSREH RfDs are shown in the table below, along with a comparison to the USEPA Health Advisories. As above, the same exposure assumptions are used by DSREH and USEPA:

	Ingestion Rate (L/kg/day)	**DSREH**		**USEPA**	
		RfD (µg/kg/day)	**Guidance (µg/L)**	**RfD (µg/kg/day)**	**Health Advisory (µg/L)**
Microcystins					
≤ 6 years	0.15	0.01	**0.07**	0.05	0.3
> 6 years	0.03	0.01	**0.3**	0.05	1.6
Cylindrospermopsin					
≤ 6 years	0.15	0.03	**0.2**	0.1	0.7
> 6 years	0.03	0.03	**1.0**	0.1	3.0
Anatoxin-a					
≤ 6 years	0.15	0.1	**0.7**	---	---
> 6 years	0.03	0.1	**3.3**	---	---

For microcystins, the guidance values recommended by DSREH are about 4-5 fold lower than those recommended by USEPA.

For cylindrospermopsin, the DSREH guidance values are about 3-fold lower than the USEPA Health Advisories for both age groups.

For anatoxin-a, DSREH developed an RfD and a guidance level, while USEPA did not.

We hope that this evaluation is helpful to your Division. Please let DSREH know if you have questions or need further assistance.

State of New Jersey
DEPARTMENT OF ENVIRONMENTAL PROTECTION
DIVISION OF SCIENCE AND RESEARCH
428 East State Street
P.O. Box 420, Mail Code 428-01
Trenton, New Jersey 08625-0420
Tel. (609) 940-4080 • Fax (609) 292-7340
www.nj.gov/dep/dsr

PHILIP D. MURPHY
Governor

SHEILA Y. OLIVER
Lt. Governor

SHAWN M. LATOURETTE
Acting Commissioner

TO: Patricia Ingelido, Director, Division of Water Supply and Geoscience

THROUGH: Gary A. Buchanan, Ph.D., Director, Division of Science and Research GB
Mingzhu Fang, Ph.D., Chief, Bureau of Risk Analysis MF

FROM: Brian Pachkowski, Ph.D., Research Scientist BP

SUBJECT: Recommendations for NJDEP drinking water guidance for saxitoxin

DATE: May 11, 2021

Summary

This memo is in response to a request from the Division of Water Supply and Geoscience (DWSG) to the Division of Science and Research (DSR) for recommendations for drinking water guidance for the cyanobacterial toxin, saxitoxin. As requested by DWSG, these recommendations for finished drinking water are derived for a 10-day exposure period, consistent with the exposure duration used in the US Environmental Protection Agency's (USEPA's) Drinking Water Health Advisories for cyanobacterial toxins[1].

Health-based drinking water concentrations, such as NJDEP's guidance values and the USEPA's Health Advisories, are developed using toxicity factors and exposure assumptions. The USEPA has not developed a Health Advisory or toxicity factor (e.g., a Reference Dose [RfD]) for saxitoxin. However, the USEPA has established exposure assumptions (i.e., exposure duration and daily drinking water ingestion rates) for use in the derivation of drinking water guidance for cyanotoxins such as saxitoxin. As part of the development of recommended NJDEP drinking water guidance for other cyanotoxins in 2017, DSR reviewed these exposure assumptions and agreed with the USEPA's conclusions[2].

At the request of the Bureau of Freshwater and Biological Monitoring, DSR developed a short-term RfD for saxitoxin, the derivation of which underwent peer review by external experts. The detailed basis for this RfD is expected to be included in the forthcoming revision to the NJDEP

[1] The 2015 USEPA drinking water Health Advisories (10-day) and corresponding health effects support documents for cyanotoxins (microcystins, cylindrospermopsin, anatoxin-a) can be found at: https://www.epa.gov/cyanohabs/epa-drinking-water-health-advisories-cyanotoxins

[2] In a memo dated September 1, 2017, from Gloria Post to Patricia Gardner, DSR provided detailed information regarding the scientific basis for USEPA's exposure duration and daily drinking water ingestion rates used for deriving Drinking Water Health Advisories for cyanobacterial toxins.

Cyanobacterial Harmful Algal Bloom (HAB) Freshwater Recreational Response Strategy document from the Bureau of Freshwater and Biological Monitoring.

As was also the case for the other NJDEP cyanotoxin RfDs, the saxitoxin RfD was developed by DSR for use in recreational exposure guidance. As such, the RfD is intended to address **short-term exposures** that might occur over a limited period, during the outdoor swimming season, and during a HAB. This RfD is **not intended to address chronic exposure**. As such, as discussed in DSR's 2017 memo, this RfD is **equally applicable to short-term drinking water exposure**, such as the 10-day exposure period envisioned in the NJDEP drinking water guidance and USEPA Drinking Water Health Advisories for other cyanobacterial toxins (e.g., microcystin, cylindrospermopsin, anatoxin-a).

In summary, DSR recommends a drinking water guidance value for saxitoxin of 0.025 µg/L for infants and children up to 6 years of age. When saxitoxin is detected in drinking water, the guidance value for infants/young children is likely to be applied, rather than the less stringent guidance value of 0.11 µg/L for older individuals (i.e., ages 6 years and older).

Development of drinking water guidance values by DSR

Health-based drinking water concentrations, such as NJDEP drinking water guidance values and USEPA Health Advisories, are developed using exposure assumptions and toxicity factors. Both the exposure assumptions and the toxicity factor relevant to the development of drinking water guidance for saxitoxin are briefly reviewed below.

Exposure assumptions

The exposure assumptions used for USEPA Drinking Water Health Advisories for cyanotoxins (microcystin and cylindrospermopsin) were used to develop the NJDEP drinking water guidance for saxitoxin. In 2017, DSR reviewed the scientific basis of these assumptions. Having agreed with these assumptions, DSR applied them in deriving and recommending drinking water guidance values for microcystins, cylindrospermopsin, and anatoxin-a.

Exposure duration

USEPA Health Advisories for cyanobacterial toxins are intended to be protective for short-term (10-day) exposure. This exposure duration is based on human exposure scenarios for cyanobacterial blooms in drinking water.

Daily drinking water ingestion rates

As part of its 2015 Health Advisories for microcystins and cylindrospermopsin, USEPA derived separate advisories for 1) bottle fed infants and children up to 6 years of age, and 2) children 6 years of age and older and adults.

The advisories for infants and children up to 6 years old are based on 90th percentile water ingestion average[3] rates for infants from 0 to 12 months old, 0.15 L/kg/day.

The advisories for children 6 years of age and older and adults are based on the 90th percentile water ingestion rate for adults 21 years of age and older, 0.034 L/kg/day.

[3] This is based on the time-weighted average of the three separate age periods within 0 to 12 months (0 to 3 months – 0.23 L/kg/day; 3 to 6 months – 0.148 L/kg/day; 6 to 12 months – 0.112 L/kg/day).

Toxicity factor

DSR derived an oral short-term RfD for saxitoxin as part of the development of recreational exposure guidance for this toxin. **This oral RfD is also applicable to short-term drinking water exposure but is not intended to address chronic exposure. DSR recommends that this RfD be used as the basis for New Jersey drinking water guidance for saxitoxin.**
The detailed basis for this RfD developed by DSR[4] can be found in the forthcoming revision to the NJDEP Cyanobacterial HAB Freshwater Recreational Response Strategy document.

RfD for saxitoxin

The USEPA has not developed an RfD for saxitoxin.

DSR derived an RfD based on case reports of paralytic shellfish poisoning (PSP) in humans, which is caused by saxitoxin and its analogues. Using statistical modeling consistent with the USEPA's benchmark dose modeling approach, Arnich and Thébault (2018) identified a dose of 0.37 µg/kg that is estimated to result in a 10% risk of showing a PSP symptom. DSR applied a composite uncertainty factor of 100, which accounts for human variability (factor of 3), exposure duration (factor of 3), and database deficiencies (factor of 10), to derive a short-term RfD of 0.0037 µg/kg/day.

Drinking water guidance value for saxitoxin recommended by DSR

The equation for deriving a drinking water guidance is given as:

Guidance value (µg/L) = RfD (µg/kg/day) ÷ Ingestion rate (L/kg/day)

Where:
RfD = 0.0037 µg/kg/day
Ingestion rate for infants and children up to 6 years old = 0.15 L/kg/day
Ingestion rate for children 6 years of age and older and adults = 0.034 L/kg/day

Based on the equation above, the drinking water guidance values for saxitoxin are:

- 0.025 µg/L (rounded from 0.0246 µg/L) for infants and children up to 6 years old
- 0.11 µg/L (rounded from 0.108 µg/L) for children 6 years of age and older and adults

Consistent with DSR's previous approach in 2017 for recommending drinking water guidance values for other cyanotoxins based on infants and children up to 6 years of age, **DSR recommends a drinking water guidance value for saxitoxin of 0.025 µg/L.**

Reference

Arnich N, Thébault A. 2018. Dose-Response Modelling of Paralytic Shellfish Poisoning (PSP) in Humans. Toxins. 10: E141.

[4] In an email dated February 4, 2021, from Gary Buchanan to recipients in Water Resource Management, a draft final saxitoxin assessment (file name "Saxitoxin assessment final draft - 4Feb2021") was attached describing the derivation of the short-term saxitoxin RfD. This basis will be included in the 2021 revision to the NJDEP Cyanobacterial HAB Freshwater Recreational Response Strategy document.

State of New Jersey
DEPARTMENT OF ENVIRONMENTAL PROTECTION
DIVISION OF SCIENCE AND RESEARCH
428 East State Street
P.O. Box 420, Mail Code 428-01
Trenton, New Jersey 08625-0420
Tel. (609) 940-4080 • Fax (609) 292-7340
www.nj.gov/dep/dsr

PHILIP D. MURPHY
Governor

SHEILA Y. OLIVER
Lt. Governor

SHAWN M. LATOURETTE
Acting Commissioner

Draft Deliberative

TO: Patricia Gardner, Assistant Commissioner, Water Resource Management

THROUGH: Gary A. Buchanan, Ph.D., Director, Division of Science and Research
Mingzhu Fang, Ph.D., Chief, Bureau of Risk Analysis

FROM: Brian Pachkowski, Ph.D., Research Scientist BP
Gloria Post, Ph.D., Research Scientist GP

SUBJECT: Clarification of exposure duration for cyanotoxin drinking water guidance values

DATE: June 23, 2021

Summary

This memo is intended to clarify issues discussed by Water Resource Management (WRM) and Division of Science and Research (DSR) at a meeting about the saxitoxin drinking water guidance value on June 17, 2021. Specifically, clarification was requested regarding application of the guidance values derived by DSR for saxitoxin and other cyanotoxins (anatoxin-a, cylindrospermopsin, microcystins) to a 30-day exposure duration instead of the 10-day exposure duration used in the USEPA Drinking Water Health Advisories (HAs) for cyanotoxins[1].

On April 12, 2021, the Division of Water Supply and Geoscience (DWSG) requested that DSR "make recommendations for guidance on saxitoxin in finished drinking [water] to be applied as the 10-day exposure period intended in the USEPA drinking water Health Advisories." In May 2021, DSR provided a memo with a recommended drinking water guidance value for saxitoxin of 0.025 µg/L[2]. Although the memo acknowledged the duration of exposure (10-day) specified in DWSG's request, DSR did not intend the saxitoxin drinking water guidance value to be explicitly applicable to only 10 days of exposure. As explained below, USEPA selected the

[1] The 2015 USEPA drinking water Health Advisories (10-day) and corresponding health effects support documents for cyanotoxins (microcystins, cylindrospermopsin) can be found at: https://www.epa.gov/cyanohabs/epa-drinking-water-health-advisories-cyanotoxins

[2] In a memo dated May 11, 2021, from Brian Pachkowski to Patricia Ingelido, DSR provided detailed information on the scientific basis (e.g., short-term Reference Dose, daily drinking water ingestion rate) for deriving the saxitoxin drinking water guidance value.

10-day exposure duration used for its cyanotoxin HAs from the exposure durations (1-day, 10-day, and lifetime) for which USEPA develops HAs in general. As USEPA HAs are non-regulatory guidance, NJDEP is not bound to these durations when implementing its drinking water guidance values.

As explained below, the NJDEP drinking water guidance values for all four cyanotoxins mentioned above and the USEPA HAs for cyanotoxins are based on short-term Reference Doses that are protective for up to 30 days of exposure. All of these NJDEP and USEPA drinking water values were derived using the same general approach. However, the NJDEP guidance and the USEPA HAs for microcystins and cylindrospermopsin are numerically different because each agency developed different short-term Reference Doses for these cyanotoxins. USEPA did not develop HAs for anatoxin-a or saxitoxin.

USEPA's 10-day Health Advisories for cyanotoxins are based on a pre-defined exposure duration

Over the past several decades, USEPA has developed HAs for a limited number of pre-defined exposure durations (1-day, 10-day, or lifetime; USEPA, 2018). Of these three options, 10 days is the most appropriate choice for cyanotoxin HAs because harmful algal blooms occur sporadically and for a relatively short duration. As such, in 2015, USEPA developed 10-day HAs for cylindrospermopsin and microcystins (USEPA, 2015a, 2015b)[3]. However, as discussed below, the fact that USEPA HAs for cyanotoxins are stated to apply to 10 days does not mean that they are not protective for somewhat longer exposure durations.

Short-term Reference Doses used for USEPA HAs and NJDEP guidance for cyanotoxins are protective for up to 30 days of exposure

In deriving health-based drinking water values in general (e.g., HAs, guidance values, Health-based MCLs), the following factors are needed: (1) Reference Doses and (2) daily drinking water ingestion rates. The Reference Dose is the parameter relevant to the applicable exposure duration for the short-term USEPA HAs and NJDEP drinking water guidance values for cyanotoxins.

Reference Doses are derived from animal toxicology or human epidemiological studies with the intent of being protective of specific exposure durations: acute (up to 24 hours), short-term (1 to 30 days), subchronic (30 to 90 days), or chronic (over 90 days)[4]. Of these, the short-term Reference Dose is most relevant to the short-term exposure duration for cyanotoxins in drinking water. As such, all of the NJDEP drinking water guidance for cyanotoxins, including the guidance for microcystins, cylindrospermopsin, and anatoxin-a developed in 2017 and the

[3] Although having prepared supporting documentation, USEPA did not derive a Health Advisory for anatoxin-a because the available data did not support the derivation of a Reference Dose needed for the advisory (https://www.epa.gov/sites/production/files/2017-06/documents/anatoxin-a-report-2015.pdf).

[4] Exposure durations are based on those in the USEPA's Integrated Risk Information System (IRIS) Glossary: https://sor.epa.gov/sor_internet/registry/termreg/searchandretrieve/glossariesandkeywordlists/search.do?details=&vocabName=IRIS%20Glossary#formTop

guidance for saxitoxin developed in 2021, as well as the USEPA HAs for microcystin and cylindrospermopsin, are based on short-term Reference Doses.

Because all of the USEPA HAs and NJDEP drinking water guidance values for cyanotoxins were derived using short-term Reference Doses, these values can be considered to be protective for up to 30 days of exposure. While USEPA selected 10 days as the most appropriate of its three available duration options for its cyanotoxin HAs, this choice of a 10-day exposure duration can be considered to be overly protective since the HAs are actually protective for a longer duration of up to 30 days.

A clarification regarding uncertainty factors for saxitoxin

The DSR PowerPoint presented at the meeting on June 17 listed the uncertainty factors for human variability, duration, and database as 3, 10, and 3, respectively. However, as noted during the June 17 discussion, the correct assignment of the uncertainty factors for human variability, duration, and database is actually 3, 3, and 10, respectively. That error has no implication on the short-term Reference Dose or drinking water guidance value for saxitoxin. These uncertainty factors are already correctly assigned in the May 2021 saxitoxin memo from DSR to DWSG and in the "2021 NJDEP Cyanobacterial Harmful Algal Bloom Freshwater Recreational Response Strategy" document (NJDEP, 2021).

References

NJDEP. 2021. 2021 Cyanobacterial Harmful Algal Bloom (HAB) Freshwater Recreational Response Strategy. https://www.state.nj.us/dep/hab/download/HAB2021StrategyFinal.pdf

USEPA. 2015a. Drinking Water Health Advisory for the Cyanobacterial Toxin Cylindrospermopsin. United States Environmental Protection Agency. Office of Water. EPA-820R15101. https://www.epa.gov/sites/production/files/2017-06/documents/cylindrospermopsin-report-2015.pdf

USEPA. 2015b. Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins. United States Environmental Protection Agency. Office of Water. EPA- 820R15100. https://www.epa.gov/sites/production/files/2017-06/documents/microcystins-report-2015.pdf

USEPA. 2018. 2018 Edition of the Drinking Water Standards and Health Advisories Tables. United States Environmental Protection Agency. Office of Water. EPA 822-F-18-001. https://www.epa.gov/sites/production/files/2018-03/documents/dwtable2018.pdf