WHO/HEP/ECH/WSH/2020.1

Cyanobacterial toxins: anatoxin-a and analogues

Background document for development of
WHO *Guidelines for drinking-water quality* and
Guidelines for safe recreational water environments

World Health Organization

WHO/HEP/ECH/WSH/2020.1

Suggested citation. Cyanobacterial toxins: anatoxin-a and analogues. Background document for development of WHO Guidelines for drinking-water quality and Guidelines for safe recreational water environments. Geneva: World Health Organization; 2020 (WHO/HEP/ECH/WSH/2020.1). Licence: CC BY-NC-SA 3.0 IGO.

Cataloguing-in-Publication (CIP) data. CIP data are available at http://apps.who.int/iris.

Preface

Access to safe drinking-water is essential to health, a basic human right and a component of effective policy for health protection. A major World Health Organization (WHO) function to support access to safe drinking-water is the responsibility "to propose ... regulations, and to make recommendations with respect to international health matters ...", including those related to the safety and management of drinking-water.

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International standards for drinking-water*. It was revised in 1963 and 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for drinking-water quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published in 1998, addressing selected chemicals. An addendum on microbiological aspects, reviewing selected microorganisms, was published in 2002. The third edition of the GDWQ was published in 2004, the first addendum to the third edition was published in 2006, and the second addendum to the third edition was published in 2008. The fourth edition was published in 2011, and the first addendum to the fourth edition was published in 2017.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation relating to aspects of protection and control of drinking-water quality is accordingly prepared and updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other information to support the GDWQ, describing the approaches used in deriving guideline values, and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a background document evaluating the risks to human health from exposure to that chemical in drinking-water was prepared. The draft health criteria document was submitted to a number of scientific institutions and selected experts for peer review. The draft document was also released to the public domain for comment. Comments were carefully considered and addressed, as appropriate, taking into consideration the processes outlined in the *Policies and procedures used in updating the WHO guidelines for drinking-water quality* and the WHO *Handbook for guideline development*. The revised draft was submitted for final evaluation at expert consultations.

During preparation of background documents and at expert consultations, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents; the International Agency for Research on Cancer; the Joint Food and Agriculture Organization of the United Nations (FAO)/WHO Meeting on Pesticide Residues; and the Joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO website and in the current edition of the GDWQ.

Acknowledgements

This background document on anatoxin-a in drinking-water for the development of the World Health Organization (WHO) *Guidelines for drinking-water quality* (GDWQ) and *Guidelines for safe recreational water environments* was prepared by Dr Andrew Humpage, University of Adelaide (formerly South Australian Water Corporation), Australia.

The work of the following experts was crucial in the development of this document and others in the second addendum to the fourth edition:

Dr M Asami, National Institute of Public Health, Japan
Dr RJ Bevan, independent consultant, United Kingdom
Mr R Carrier, Health Canada, Canada
Dr J Cotruvo, Joseph Cotruvo & Associates and NSF International WHO Collaborating Centre, United States of America
Dr D Cunliffe, South Australian Department of Health, Australia
Dr L D'Anglada, Environmental Protection Agency, United States of America
Dr A Eckhardt, Umweltbundesamt (Federal Environment Agency), Germany
Professor JK Fawell, Cranfield University, United Kingdom
Dr A Hirose, National Institute of Health Sciences of Japan
Dr A Humpage, University of Adelaide (formerly South Australian Water Corporation), Australia
Dr P Marsden, Drinking Water Inspectorate, United Kingdom
Professor Y Matsui, Hokkaido University, Japan
Professor CN Ong, National University of Singapore, Singapore
Dr E Testai, National Institute of Health, Italy

The draft text was discussed at expert consultations held on 14–16 July 2016, 28–30 March 2017 and 13–14 July 2018 on the second addendum to the fourth edition of the GDWQ or *Toxic cyanobacteria in water*. Input from all those who participated in these meetings is gratefully acknowledged. The final version of the document also takes into consideration comments from both peer reviewers and the public, as well as other experts who contributed to the text. These include I Chorus, formerly Federal Environment Agency of Germany; I Falconer, University of Adelaide, Australia; J Fastner, Federal Environment Agency of Germany; A Foss, Greenwater Labs, United States of America; L Lawton, Robert Gordon University, Scotland; J Puddick, Cawthron Institute, New Zealand; B Lampe, NSF International, United States of America; and M Welker, independent consultant, Germany.

The coordinator was Ms J De France, WHO, with support from Dr V Bhat, formerly of NSF International, United States of America. Strategic direction was provided by Mr B Gordon, WHO. Dr A Tritscher, formerly of WHO, and Dr P Verger, WHO, provided liaisons with the Joint FAO/WHO Expert Committee on Food Additives and the Joint FAO/WHO Meeting on Pesticide Residues. Dr R Brown and Ms C Vickers, WHO, provided liaisons with the International Programme on Chemical Safety. Dr M Perez contributed on behalf of the WHO Radiation Programme. Dr Andina Faragher, Biotext, Australia, was responsible for the scientific editing of the document.

Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document are greatly appreciated.

Acronyms and abbreviations

ALF	alert level framework
ATX	anatoxin-a
bw	body weight
dhATX	dihydroanatoxin-a
dhHTX	dihydrohomoanatoxin-a
GV	guideline value
HTX	homoanatoxin-a
i.p.	intraperitoneal
LD_{50}	median lethal dose
nAChR	nicotinic acetylcholine receptor
NOAEL	no-observed-adverse-affect level
TCiW	*Toxic cyanobacteria in water* (WHO guidebook)
WHO	World Health Organization

Contents

Information on cyanobacterial toxins, including anatoxin-a, is comprehensively reviewed in a volume to be published by the World Health Organization, *Toxic cyanobacteria in water* (TCiW; Chorus & Welker, in press). TCiW covers chemical properties of the toxins and information on the cyanobacteria that produce them, as well as guidance on assessing the risks of toxin occurrence, monitoring and management. In contrast, this background document focuses on reviewing toxicological information and other considerations for deriving guideline values for anatoxin-a in water. Sections 1, 2 and 7 are largely summaries of respective chapters in TCiW, and readers are referred to corresponding chapters in TCiW for further information, including references to original publications.

Executive summary

Anatoxin-a (ATX) and its analogues (ATXs) are alkaloids produced by strains of various species of cyanobacteria found primarily in freshwater environments. Many of these species are benthic (i.e. grow on sediments or other submerged surfaces). ATXs have often been linked to deaths of dogs and wild animals. Drinking-water is the most likely exposure route for humans. Recreational activities in lakes with cyanobacterial blooms may also intermittently expose individuals to high concentrations of ATX. Food is not considered a significant source of exposure, but limited data are available.

The main driver of high amounts of cyanobacterial biomass is nutrients from anthropogenic sources such as agricultural runoff and wastewater. Hence, control of these sources is the primary long-term management option. Drinking-water can usually be treated to acceptable levels by a well-run conventional treatment plant implementing coagulation, flocculation and filtration; chlorination is not sufficiently effective against ATX. If this is not sufficient, ozonation and activated carbon filtration or addition of powdered activated carbon can be effective.

The (+)-ATX enantiomer binds with high affinity to nicotinic acetylcholine receptors of nerve cells, causing chronic overstimulation. This can lead to increases in heart rate and blood pressure, as well as fatigue and eventual paralysis of muscles, which can cause death when it occurs in respiratory muscles. Although ATX is the best studied analogue, limited evidence suggests that homoanatoxin-a (HTX) and the dihydro derivatives of ATX and HTX bind to the same receptor and may have similar potency to ATX when administered orally.

The toxicological database on ATXs is not adequate to support derivation of a formal guideline value. Nevertheless, a "bounding value" may be useful to risk assessors. Based on the limited available studies of acute and subchronic (28 days) ATX toxicity, provisional health-based reference values are provided that are unlikely to cause adverse effects in exposed adults. These values are 30 µg/L for acute or short-term exposure via drinking-water and 60 µg/L for recreational water exposure. There are no studies of chronic ATX exposure, and so a value for lifetime exposure cannot be calculated. Given the evidence that the analogues mentioned above are of similar toxicity to ATX, it is recommended that they be included in calculations of total ATXs – as gravimetric or molar equivalents – when evaluating against the provisional health-based reference values.

The health-based reference values are derived for adults. As a result of their higher water consumption per unit body weight, it is recommended, as a precautionary measure, that bottle-fed infants and small children be provided with an alternative water source if ATX concentrations are greater than 6 µg/L for short periods.

1 General description

1.1 Identity

Anatoxin-a (ATX), or 2-acetyl-9-azabicyclo[4:2:1]non-2-ene, is a tropane-related bicyclic, secondary amine alkaloid that is produced by a number of species of cyanobacteria. ATX and its analogue homoanatoxin-a (HTX) have almost identical toxicological properties. Fig. 1.1 shows that ATX differs from HTX in the presence of an additional methyl group (CH) on carbon atom 11 (C11). Dihydroanatoxin-a (dhATX) and dihydrohomoanatoxin-a (dhHTX) are synthesized by some cyanobacterial species in greater amounts than ATX and HTX (Heath et al., 2014; Méjean et al., 2016), but may also be formed in the environment by reduction of ATX and HTX, respectively (Mann et al., 2012). Other derivatives of ATX have been identified in cyanobacterial cultures or in field samples that may be degradation products or partially synthesized compounds – they include 2,3-epoxy-, 4-hydroxy- and 4-oxo- derivatives (*Toxic cyanobacteria in water* [TCiW], Testai, in press).

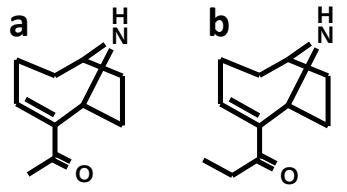

Fig. 1.1. Structures of (a) anatoxin-a and (b) homoanatoxin-a

1.2 Physicochemical properties

Known physicochemical properties of ATX and HTX are summarized in Table 1.1. ATX is highly soluble in water, with saturation concentrations several orders of magnitudes higher than the World Health Organization (WHO) provisional health-based reference values (see section 8.1). Limited information is available on chemical breakdown, biodegradation and distribution in the environment (TCiW, Testai, in press).

The pKa of the synthesized (+)-ATX enantiomer is 9.36, indicating that the molecule is protonated at physiological pH (Koskinen & Rapoport, 1985).

Table 1.1. Properties of anatoxin-a and homoanatoxin-a

Property	Anatoxin-a	Homoanatoxin-a
CASRN	64285-06-9	142926-86-1
Chemical formula	$C_{10}H_{15}NO$	$C_{11}H_{17}NO$
Average molecular weight[a] (g/mol)	165.237	179.264
Monoisotopic mass[b] (Da)	165.115	179.131
Boiling point (°C)	291	NA
K_{ow}[c]	0.8	1.3

CASRN: Chemical Abstracts Service Registry Number; NA: not applicable

[a] Average molecular weight calculated based on conventional atomic weights as given in Table 3 of Meija et al. (2016)

[b] Calculated based on atomic mass of isotopes given by NIST (2019); rounded to three digits

[c] K_{ow} computation with XLOGP3 (Cheng et al., 2007)

1.3 Organoleptic properties

None of the known cyanobacterial toxins (microcystins, cylindrospermopsins, saxitoxins, ATXs) have been shown to affect the taste or odour of water. However, some cyanobacterial species produce other compounds, such as geosmin and methyl-isoborneol, that do affect taste and odour, indicating the presence of cyanobacteria in raw water. As this applies only to some strains of some cyanobacterial species, the absence of these typical tastes and odours is not a reliable indicator of the absence of cyanotoxins. For an overview of the relationship between organoleptic properties and toxins, see TCiW, Kaloudis (in press).

1.4 Major uses and sources

ATXs occur naturally, although high concentrations are typical for fresh waters influenced by human activity – for example, by effluents from wastewater or runoff from agricultural land that introduces nutrients that fertilize the growth of phototrophic organisms, including cyanobacteria. There are no known commercial applications of ATXs. ATXs are produced by strains of various species within a number of cyanobacterial genera – in particular, *Anabaena* (some species of which are now classified as *Dolichospermum*), *Aphanizomenon* (some species of which are now classified as *Cuspidothrix* and some as *Chrysosporum*), *Raphidiopsis* (formerly *Cylindrospermopsis*), *Cylindrospermum*, *Oscillatoria*, *Planktothrix*, *Phormidium*, *Lyngbya* (some species of which are now classified as *Microseira* and some as *Moorea)*, *Tychonema*, *Blennothrix* and *Kamptonema*. For more information on the new classification of genera, see TCiW, Vidal et al. (in press). Producing and nonproducing strains are known for all species for which ATX production has been observed.

Production of ATX is both species and strain specific. ATX is co-produced with HTX, dhATX and/or dhHTX in varying shares. A few cyanobacterial strains have been reported to produce both microcystins and ATX. The ATX content per cell (i.e. "cell quota") of individual strains generally varies 2–7-fold, depending on growth conditions and environmental factors. ATX contents are not consistently related to cell growth phases.

From the limited available data, cell quotas for ATX/HTX range from trace amounts up to 500 fg/cell in *Phormidium* spp. Toxin contents of up to 13 mg/g dry weight were reported from *Dolichospermum* (formerly *Anabaena*) spp. and *Oscillatoria* spp. Toxin contents of other genera were 1–2 orders of magnitude lower.

Biosynthesis of the ATXs involves polyketide synthases. Complete gene cluster sequences (*anaA-G*, approximately 25 kbp) have been determined for strains from several genera (*Oscillatoria*, *Cylindrospermum*, *Cuspodothrix*), and the individual steps of the biosynthesis have been studied. Information on the molecular regulation of biosynthesis is largely lacking.

For more details on ATX-producing organisms and biosynthesis, see TCiW, Testai (in press).

2 Environmental levels and human exposure

2.1 Air

ATXs are not volatile. Exposure via inhalation is possible only through spray carrying cyanobacterial cells or toxins – for example, via overhead irrigation, during storms or in the wake of a power boat. No data on inhalation exposure or concentrations in sprays were found.

2.2 Food

Studies of ATX concentrations in fish, shellfish and edible crops are scarce. The reliability of some of the methods used in earlier studies is under question (Testai et al., 2016). There are also few analyses of ATXs in dietary supplements, and the concentrations reported range from non-detectable up to 33 µg/g. There is no evidence of bioaccumulation of ATX.

For more information on ATX in food and dietary supplements, see TCiW, Ibelings, Foss & Chorus (in press), and Dietrich (in press).

2.3 Water

Since ATX-producing cyanobacteria are found primarily in freshwater environments, in many settings the primary waterborne route of human exposure to ATXs is the consumption of drinking-water, if it is produced from surface waters that are untreated or insufficiently treated. Another exposure route – important in some settings – is the recreational use of lakes and rivers. Depending on the seasonal patterns of cyanobacterial blooms and water body use, patterns of exposure may be episodic. Although cyanobacteria that could potentially produce ATXs occur widely in diverse freshwater environments around the globe, with the exception of a few locations (e.g. in New Zealand), ATXs have generally been detected less frequently than microcystins and cylindrospermopsin.

Concentrations rarely exceed tens of µg/L in open water, but have been reported to exceed 1000 µg/L in surface blooms. Blooms of some planktonic ATX-producing cyanobacteria (e.g. *Dolichospermum*) show an intermediate tendency to form surface scums. Blooms of *Planktothrix agardhii* occur in well-mixed, shallow water bodies and only rarely form light surface blooms, resulting in spatially more homogeneous ATX concentrations.

Only a few reports on ATX in drinking-water are available, with concentrations generally in the low µg/L range; up to 8.5 µg/L have been reported. Among ATX-producing species, several do not form water blooms but occur as benthic mats in rivers (i.e. on the sediment surface) or periphyton (attached to higher aquatic plants), resulting in a highly patchy distribution of ATXs on such surfaces. Intoxications of pet dogs or livestock have been reported after ingestion of periphyton on lumps of detached aquatic plants or scum containing high amounts of ATX. Only limited and inconclusive information is available on the release of ATXs from cells and its persistence in surface waters. Available data, however, indicate that ATXs are largely confined to viable cells and released primarily through cell lysis, followed by rapid degradation.

Recreational activity in surface waters with cyanobacterial blooms can cause exposure to ATXs (and other toxins in blooms and scums), mainly through unintentional swallowing of water. Recreational activity typically takes place at near-shore sites where surface blooms or scums accumulate, and where benthic cyanobacteria grow. Contact with scum material may potentially expose people to high concentrations of ATXs, but human uptake is unlikely for detached aquatic plants with toxic periphyton.

Inhalational exposure may be a relevant pathway for specific recreational activities, such as waterskiing or jet-skiing, and for specific occupational situations involving spraying with water containing bloom material, such as spray irrigation or dust suppression. Therefore, recreational activity and in some cases occupational activity may be a potentially substantial exposure route, although in most cases for a limited time.

For more information on occurrence of ATXs in the environment and drinking-water, see TCiW, Testai (in press).

2.4 Estimated total exposure and relative contribution of drinking-water

As for the other cyanotoxins, where surface water is used as the source for drinking-water, this is the most likely means of exposure to ATXs. There is no indication of food being a significant source of oral exposure, but available data are very limited. Recreational activities in lakes with cyanobacterial blooms may intermittently expose individuals to high concentrations of ATX, as described in section 2.3. For most situations for the general population, the oral route is the main route of concern.

Patterns and duration of exposure are strongly influenced by region and lifestyle. Estimating total exposure or the relative contribution of particular exposure routes (e.g. food, drinking-water) requires specific analyses of ATX concentrations in samples from the respective media in a given setting. Chapter 5 of TCiW (in press) gives further guidance and background information on assessing routes of exposure.

For specific population groups, exposure may occur via the parenteral route – for example, associated with use of contaminated water for haemodialysis or infusions. Risks are potentially high if water from contaminated surface waters is used for haemodialysis, which was the major source of exposure to other cyanotoxins (including some lethal exposures) documented in the cases discussed in TCiW, Azevedo (in press).

A specific aspect of exposure to ATXs, compared with other cyanotoxins, is the potential for high concentrations in beached or floating benthic mat material, or macrophytes (submerged aquatic plants) associated with ATX-producing cyanobacteria. ATX concentrations appear to be high only in the direct vicinity of such material. However, animal fatalities resulting from ingestion of this material give rise to concern about direct human contact and possible unintentional ingestion. Contact with such material should therefore be avoided.

3 Kinetics and metabolism in laboratory animals and humans

3.1 Absorption

In acute toxicity studies in animals, signs of neurotoxicity, including loss of coordination, muscular twitching and death from respiratory paralysis, occur within minutes of oral exposure (Stevens & Krieger, 1991; Fitzgeorge, Clark & Keevil, 1994). This indicates that ATX is rapidly absorbed from the gut following oral exposure.

3.2 Distribution

Specific studies designed to analyse tissues for ATX distribution were not identified. However, as with absorption, the rapid appearance of symptoms following oral exposure suggests widespread distribution of ATX to the central and peripheral nervous systems, where nicotinic acetylcholine receptors (nAChRs) are located. As reviewed by Wonnacot & Gallagher (2006), secondary amines such as ATX can cross the blood–brain barrier to interact with nAChRs; this is supported by the observed neurological symptoms after exposure.

3.3 Metabolism

No published studies were identified that have investigated in vivo metabolism of ATX in mammals. A study in rainbow trout fry (*Oncorhynchus mykiss*) injected via the intraperitoneal

(i.p.) route with sublethal doses of (±)-ATX (0.08–0.31 mg/g) showed increases in liver ethoxyresorufin-*O*-deethylase and glutathione *S*-transferase activity. This suggests some interaction with both phase I and phase II metabolic pathways (Osswald et al., 2013), although not necessarily linked to direct involvement in ATX biotransformation.

3.4 Elimination

It appears that at least some ATX is excreted unchanged in urine and bile, since it was detected in these fluids from a dog poisoned by toxic *Phormidium* (Puschner, Pratt & Tor, 2010). However, the analytical method used (ion trap mass spectrometry) was not specifically targeted to detect metabolites (Puschner, Hoff & Tor, 2008).

4 Effects on humans

Little is currently known about ATX exposure and subsequent effects on humans. A reported case of suspected human poisoning by ATX occurred in a 17-year-old boy who died 2 days after swallowing water while swimming in a golf course pond containing a *Dolichospermum* (formerly *Anabaena*) *flos-aquae* bloom. However, a chromatographic peak with the retention time and molecular mass of ATX from samples of liver, blood and fluids collected postmortem was later identified as phenylalanine (TCiW, Carmichael, in press).

In a review of 11 disease outbreaks reported to be associated with cyanobacterial blooms in the United States of America in 2009–2010, ATX (0.05–15 µg/L) was detected in three of four outbreaks where testing for ATX was conducted. However, in all three cases, microcystins were also detected (at 0.3, 4.6 and >2000 µg/L). Cylindrospermopsin was present in two cases (at 0.3 and 9 µg/L), and saxitoxin was reported in one case (at 0.09 µg/L) (Hilborn et al., 2014). Although multiple toxins were detected, it is noteworthy that neurological symptoms were reported in all three ATX-associated outbreaks but in none of the other outbreaks.

5 Effects on experimental animals and in vitro systems

5.1 Acute exposure

Deaths of dogs, livestock and wild animals due to poisoning by ATXs have been reported many times throughout the world, including in France, Ireland, the Netherlands, New Zealand, Scotland and the USA (Testai et al., 2016; Health Canada, 2018). In many cases, the producing cyanobacteria were benthic species, detached and washed ashore, and dogs had consumed lumps of material. Planktonic species can also stick to an animal's fur, to be licked off upon grooming. These circumstances may increase the chance of ingestion of a lethal dose by an animal. Because an acute dose of ATX will kill within minutes, the cadaver is likely to be found next to the water body, allowing the causal association with ATX to be made. Although ATX has been detected in the stomach contents and other tissues of poisoned dogs at necropsy, no estimates of a lethal dose have been made from these case studies.

In mice, the acute oral median lethal dose (LD_{50}) of a synthetic (+)-ATX preparation was 13.3 mg/kg body weight (bw) (95% CI = 12.8–14.1), and the acute i.p. LD_{50} was 0.21 mg/kg bw (95% CI = 0.20–0.24) (Stevens & Krieger, 1991). However, when ATX was derived from cyanobacterial extracts, some preparations were 2–3 times more potent via the oral route than expected from their ATX content or i.p. potency (Stevens & Krieger, 1991; Fitzgeorge, Clark & Keevil, 1994). The reason for this discrepancy is not known, but it suggests that ATX content of cyanobacterial extracts may not be an accurate predictor of oral potency. The congener dhATX, not analysed by these authors, has been suggested as being most likely

responsible for some dog deaths (Wood et al., 2017). Furthermore, a recent study indicates that dhATX was about 4-fold more toxic than ATX when administered to female Swiss albino mice by gavage (Puddick et al., 2021). Exposure to a lethal dose either orally or i.p. caused death by respiratory paralysis within a few minutes.

The acute i.p. toxicity of HTX was reported to be similar to that of ATX (i.p. LD_{50} = 0.25 mg/kg bw in mice), producing the same symptoms and death within 7–12 minutes (Skulberg et al., 1992). When given orally by gavage, an HTX-containing extract was 10 times less toxic than a similar dose administered via the i.p. route (Lilleheil et al., 1997).

5.2 Short-term exposure

Astrachan, Archer & Hilbelink (1980) conducted a 54-day drinking-water study in female Sprague–Dawley rats. ATX was partially purified from cultures of *Dolichospermum* (formerly *Anabaena*) *flos-aquae* strain NRC-44-1, with the final concentration determined by molar absorptivity. Groups of 20 rats were exposed to 0, 0.51 or 5.1 ppm ATX in drinking-water; the estimated daily exposures were 0, 0.05 and 0.5 mg/kg bw, respectively (US EPA, 2015). Food consumption and body weights were monitored, and haematology, serum enzymes, histopathology and liver mixed function oxidase activities were analysed at the end of the study. No adverse effects were seen in any animal.

Fawell et al. (1999) conducted a 5-day repeated gavage dosing trial in Crl:CD-1(ICR)BR (VAF plus) mice to determine a maximum tolerated dose for a 28-day study. Doses of (+)-ATX HCl used in the 5-day trial were 1.5, 3.0, 7.5 and 15 mg/kg bw. All animals in the highest dose group and one in the 7.5 mg/kg bw dose group died within 5 minutes of dosing, so 3.0 mg/kg bw was chosen as the highest dose for the 28-day study. The 28-day study used four dose groups of 10 mice of each sex dosed daily by gavage with (+)-ATX HCl at 0, 0.12, 0.6 or 3.0 mg/kg bw (equivalent to doses of pure (+)-ATX of 0, 0.098, 0.49 or 2.46 mg/kg). Body weight, food consumption and signs of illness were monitored in all mice through the trial, and detailed histopathology, haematology and serum biochemistry analyses were conducted for control and high-dose animals at the end of the study. One mouse in each of the highest two dose groups died within 2.5 hours of dosing. Necropsy did not show the cause, meaning that ATX toxicity could not be excluded. No other treatment-related effects were seen in any animal for any parameter examined. The authors therefore designated 0.098 mg/kg bw of pure (+)-ATX as the no-observed-adverse-effect level (NOAEL), but noted that the NOAEL could actually be 2.46 mg/kg bw.

No long-term studies on the systemic effects of ATX were identified.

5.2.1 Neurological effects

Since ATX is a neurotoxin, the primary effects are related to the nerves and the muscles they control. Two daily i.p. injections of ATX at 0.21 mg/kg bw or higher in mice caused decreased motor activity, altered gait, difficulty breathing and convulsions 5–6 minutes post-exposure, and death within 10 minutes. Less severe clinical signs were observed in animals that survived lower doses, with recovery after 15–20 minutes (Rogers et al., 2005). Locomotor activity was reduced in rats by a single subcutaneous injection of ATX at 0.06–0.225 mg/kg bw, and tolerance did not develop after a regime of four weekly injections (Stolerman, Alburquerque & Garcha, 1992; MacPhail, Farmer & Jarema, 2007). In contrast, tolerance was seen in behavioural responses in trained rats given four weekly subcutaneous injections of 0.05–0.1 mg/kg bw, but not at 0.2 mg/kg bw (Jarema, Poling & MacPhail, 2008).

5.2.2 Reproductive and developmental effects

Time-mated female mice were administered 2.46 mg/kg bw by daily gavage on gestation days 6-15. No adverse effects were noted in either the dams or their offspring (Fawell et al; 1999). In pregnant mice injected i.p. with 0.125 or 0.2 mg/kg bw on gestation days 8–12 or 13–17, reduced motor activity was noted in dams given the higher dose. However, no significant postnatal effects were observed in pups from any treatment group (Rogers et al; 2005). Treatment of pregnant golden hamsters (*Cricetus auratus*) with three i.p. doses of ATX at 0.125 or 0.2 mg/kg bw on gestation days 8–11 or 12–14 did not cause any malformations or additional resorptions, but did cause stunting at all dose levels and periods (9–24% reduction in fetus weights compared with controls) in 10–20% of fetuses (Astrachan, Archer & Hilbelink, 1980). Seven daily i.p. injections of ATX (50, 100 or 150 µg/kg bw/day) in male mice caused significant reductions in sperm count, as well as a range of other adverse effects in the testes (Yavasoglu et al., 2008).

More research on the developmental and reproductive effects of orally administered ATX is needed. As existing developmental toxicity data were derived from i.p. studies with limited relevance to oral exposure, additional oral studies are needed to adequately address this end-point.

5.2.3 Immunological effects

No data on the immunotoxicity of ATX in mammals are available.

5.2.4 Genotoxicity and carcinogenicity

(+)-ATX fumarate (0.312, 0.625, 1.25, 2.5, 5 or 10 µg/mL) was not mutagenic in any of six strains of *Salmonella* Typhimurium tested (Sieroslawska, 2013). No genotoxicity studies have been conducted using mammalian cell lines, and no in vivo carcinogenicity studies on ATX were identified.

5.3 In vitro systems

(+)-ATX HCl (4 µg/mL) caused significant reductions in cell viability (lactate dehydrogenase leakage, MTT reduction), and increases in apoptosis and DNA fragmentation in cultured Vero cells and primary rat thymocytes. Levels of reactive oxygen species increased in treated thymocytes (Rao et al., 2002).

5.4 Mode of action

ATX is a potent pre- and post-synaptic depolarizing agent. It acts by binding with high affinity to the nAChRs of motor neurons, stimulating muscle cell contraction, and neurons in the central nervous system (Aronstam & Witkop, 1981; Campos et al., 2006). Agonist binding opens the nAChR pore, allowing cations to flow into the cell, followed by automatic pore closure and a resting/desensitized state. ATX efficiently competes for nAChRs with the natural neurotransmitter acetylcholine (being 100 times more selective), which results in a more potent contractive action and overstimulation of the muscle (Swanson et al. 1986; Fawell et al. 1999). The binding is stereoselective, with a preference for (+)-ATX (Spivak et al., 1983; Macallan et al., 1988; Swanson et al., 1991).

Neuronal and peripheral nAChR interactions were investigated in vitro for 18 ATX analogues using competitive binding assays (Swanson et al., 1991; Wonnacott et al., 1991). ATX was the most potent analogue.

As well as having higher affinity for nAChRs than acetylcholine (Swanson et al., 1986), ATX is not degraded by acetylcholine esterase, so the ATX stimulatory signal to the muscle cells is not switched off, allowing prolonged interactions with nAChRs. At concentrations in the range 3.3–33 µg/L, ATX activates nAChRs and depolarizes muscle membranes (Spivak et al., 1983). At higher ATX concentrations (>130 µg/L), a neuromuscular blockade caused by receptor desensitization has been observed in vivo (Spivak, Witkop & Albuquerque, 1980; Kofuji et al., 1990). The muscle cells become fatigued and eventually paralysed, ultimately leading to respiratory failure in mammals (Carmichael, Biggs & Gorham, 1975; Valentine, Schaeffer & Beasley, 1991). Death occurs when the muscles involved in respiration are affected in this way.

ATX also affects nAChRs in the cardiovascular system to increase blood pressure and heart rate, and in the brain (Health Canada, 2018).

HTX has very similar toxicological properties and mode of action to (+)-ATX (Lilleheil et al., 1997).

6 Overall database and quality of evidence

6.1 Summary of health effects

ATX has high acute oral toxicity, with an LD_{50} of 13 mg/kg bw in mice (Stevens & Krieger, 1991). ATX chronically stimulates nAChRs in peripheral nerves, leading to muscular twitching, fatigue and paralysis. Severe overstimulation of respiratory muscles results in respiratory arrest and death within minutes. No chronic dosing studies have been conducted. In a 28-day study, a NOAEL of 0.098 mg/kg bw/day for (+)-ATX was identified, due to the unexplained death of a single mouse in each of the two highest dose groups (n = 20 per group). If these deaths are ignored, the NOAEL would be 2.46 mg/kg bw/day, indicating high uncertainty regarding the NOAEL of 0.098 mg/kg bw/day (Fawell et al., 1999). However, lethality is not generally considered an appropriate end-point for deriving a reference value. In a 54-day drinking-water study in rats, the highest dose of partially purified ATX of 0.51 mg/kg bw/day (as estimated by US EPA, 2015) did not cause any adverse effects (Astrachan, Archer & Hilbelink, 1980). In the neurodevelopmental study by Rogers et al. (2005), the NOAEL for maternal toxicity was 0.125 mg/kg bw/day for reduced motor activity seen at 0.2 mg/kg bw/day, but this value is affected by the route of administration (i.p., known to give rise to higher toxicity than the oral route) and the use of a racemic mixture (no relative content of the two stereoisomers was given, and purity was only 90%).

6.2 Quality of evidence

The overall quality of the database on ATX toxicity is very low. The available toxicological information has a large number of deficiencies, including lack of oral repeat dosing studies into sublethal effects (including locomotor, behavioural, neurodevelopmental and reproductive outcomes); lack of kinetic studies, including metabolism; lack of chronic exposure studies; and lack of robust toxicological data on HTX, or natural mixtures of ATX with its congeners or degradation products.

The reliability of the studies that are available is also low. There are relatively few oral studies or studies that used well-characterized (usually synthetic) (+)-ATX. Many oral studies used

partially purified preparations, some of which have shown discrepancies between the expected potency (based on known ATX content) and the actual potency in animals. None of the subchronic dosing studies included verification of the stability of ATX in the dosing solutions, despite the known instability of ATX in light. Lastly, most animal studies available for risk assessment included analysis of end-points such as histopathology, haematology and serum chemistry, which may not be strongly affected by a neurotoxin.

The only conclusion about the toxicity of ATX that can be made with confidence is that it is a potent and fast-acting neurotoxin that chronically activates nAChRs, leading to overstimulation of muscles, which results in muscle fatigue and paralysis. When this occurs in the respiratory muscles, death ensues.

7 Practical considerations

ATXs have been less frequently reported in lakes and reservoirs than other cyanobacterial toxins such as microcystins and cylindrospermopsin. Persistent planktonic blooms of ATX-producing cyanobacteria with health-relevant concentrations of ATXs do not appear to be common. However, benthic cyanobacteria producing ATXs have been reported in several countries. The often patchy occurrence of benthic or periphytic species hampers systematic monitoring of entire water bodies. Lethal intoxications of animals (both wild and domestic) have been attributed to ATXs, including from benthic species. These can cause considerable public health concern, requiring investigation, risk assessment and possibly protective action.

Chapters 7–10 of TCiW (in press) give guidance on multiple barriers to reduce cyanotoxin levels in water, including controlling nutrient loads from the catchment, managing water bodies, optimizing sites for drinking-water off-takes or recreation, applying drinking-water treatment to remove cyanobacteria and cyanotoxins, and providing information or warnings for recreational use of water bodies with blooms. This includes guidance on planning, managing and documenting the measures used to mitigate cyanotoxin risks by developing a water safety plan (Bartram et al., 2009; TCiW, Chorus & McKeown, in press).

7.1 Monitoring

Depending on a range of conditions, including climate, cyanobacteria can be present in surface waters at low numbers throughout the year or as short-lived seasonal blooms; in both cases, they may produce significant concentrations of toxins. Monitoring of source waters should include assessing for factors that can affect the growth of cyanobacteria, including total phosphorus, temperature, water residence time and pH (for details, see TCiW, Padisák et al., in press). On-site visual assessment for turbidity with greenish discolouration or scums – for example, using a Secchi disc to measure water transparency – and microscopy are effective, low-cost, direct methods that can trigger increased vigilance if ATX-producing cyanobacteria are observed. Monitoring over several seasons can often establish the likely occurrence and timing of favourable conditions for cyanobacterial growth, as well as the taxonomic composition and magnitude of blooms. For example, a lake with regular seasonal blooms of *Aphanizomenon* in late summer is unlikely to shift to perennial blooms of *Raphidiopsis* from one year to the next (TCiW, Ibelings, Foss & Chorus, in press).

Monitoring programmes should be adaptive, so that sampling and analyses are increased when there is evidence of increasing amounts of cyanobacteria. For early warning and to trigger short-term management responses, alert level frameworks (ALFs) are useful both for drinking-water and for recreational water use. To trigger alerts, these frameworks primarily use levels

of cyanobacterial biomass (measured as biovolume or chlorophyll *a*; Table 7.1) below which concentrations exceeding the health-based values of cyanotoxins for drinking-water (acute, short-term or lifetime) or recreational water are unlikely.

Table 7.1. Alert levels for cyanobacterial biomass indicators that trigger management responses

Alert level	Indicators of cyanobacterial biomass	
	Biovolume (mm^3/L)	**Chlorophyll *a* (with cyanobacteria dominant) (µg/L)**
Alert Level 1 threshold for drinking-water	0.3	1
Alert Level 2 threshold for drinking-water	4	12
Alert level threshold for recreational water use	8	24

As described in the ALF, monitoring of source waters can start with simple site inspections for appearance of visible blooms, assessing transparency using a Secchi disc. However, not all ATX producers form surface scums or strong discolouration; those that do not may be overlooked. Therefore, if the presence of cyanobacteria is suspected, microscopic examination for the presence of cyanobacteria that could potentially produce ATXs is important. As blooms develop, monitoring can be expanded to include quantitative measures of cyanobacterial biomass that could indicate potential toxin concentrations, such as cyanobacterial biovolumes or chlorophyll *a*, or direct analyses of ATX concentrations.

Wherever possible, toxin analyses (ideally including ATX, dhATX, HTX and dhHTX) should be performed if ATXs are suspected. This is because concentrations associated with blooms can vary substantially. The data from toxin analyses may allow restrictions on site use to be avoided or lifted where these were based on biovolume or chlorophyll *a* concentrations.

Template alert level decision trees for monitoring cyanobacteria and responding to exceedances are given in TCiW, Humpage & Cunliffe (in press) for drinking-water and in TCiW, Chorus & Testai (in press) for recreational water exposure.

ATXs can be associated with benthic mats or floating clumps of detached underwater vegetation that have a patchy and ephemeral occurrence. Testing for ATXs should have high priority if such material is considered an issue in individual water bodies. In several reported cases, analysis of ATXs followed incidents of animal intoxication.

7.2 Analytical methods and achievability

Analytical techniques are available for the range of parameters associated with cyanobacterial blooms and ATXs. The complexity, expertise requirements and costs of monitoring vary. Techniques range from relatively simple visual inspections; to testing for phosphorus, pH, Secchi disc transparency, cell numbers, species identification, biovolumes and chlorophyll *a*; to toxin analysis. Limits of quantification well below 30 µg/L can be achieved with all of the established methods outlined below. For less sensitive detection methods, appropriate sample concentration is required. More sensitive methods generally require less sample preparation, but costs per analysis tend to be higher.

For cell-bound and total (cell-bound plus extracellular) ATXs, extraction (e.g. by freeze–thaw cycles) is performed before analysis. Filtration can be used to separate cells so that intracellular and extracellular fractions can be tested separately. ATXs can be effectively extracted from cells by acidified water or acidified mixtures of methanol and water. Solid-phase extraction using graphitized carbon or a weak cation exchanger can be used to concentrate ATXs to achieve lower detection limits or for sample clean-up.

Among the methods available for analysing ATXs, high performance liquid-chromatography (HPLC) coupled with post-derivatization fluorescence or tandem mass spectrometry are the most specific. Both methods require some knowledge about the derivatives of ATXs so that they can be targeted; higher resolution is achieved with mass spectrometry techniques. US EPA Method 545, based on liquid chromatography – tandem mass spectrometry (LC-MS/MS) for ATXs has a reported detection limit of 0.049 µg/L (Shoemaker & Dietrich, 2017). Certified reference material for ATX and dhATX are commercially available. Synthesized ATX is also readily available for purchase as a hydrochloride or fumarate salt, requiring correction for mass differences. Most of these products are not enantiomerically separated and are prepared as a racemic (±) mixture. The (+)-ATX form extracted from cyanobacteria is also available.

ELISA (enzyme-linked immunosorbent assay) and RBA (receptor binding assay) kits are also commercially available, which detect both ATX and HTX (and probably other variants), with reported detection limits of 0.15 µg/L for ATX and 10 µg/L for HTX.

These methods were primarily developed for the analysis of water samples. Their application to more complex matrices (e.g. food, stomach/tissue contents) requires identification of matrix effects, prior clean-up, and determination of recovery rates by spiking samples with known amounts of ATXs.

Molecular tests have been developed to identify the presence of one gene fragment involved in the production of ATX for various cyanobacterial taxa. These methods do not provide information about actual toxin production or concentrations, but can provide early warning of potential occurrence (see TCiW, Padisák et al, in press, for further information).

The choice of analytical methods depends on local or regional accessibility, costs and, in particular, the purpose of the analyses. Objectives may include screening for risk assessment and research.

For more information on analytical methods, see TCiW, Lawton et al. (in press).

7.3 Source control

For planktonic toxic cyanobacteria, preventing blooms in source waters is the key to long-term control of the risks they present. The most sustainable approach is to keep concentrations of plant nutrients low. Most cyanobacteria proliferate under eutrophic conditions – that is, elevated concentrations of nutrients, especially phosphorus – and total phosphorus concentrations below 20–50 µg/L (with the threshold depending on water body characteristics) will often limit the development of cyanobacterial blooms (TCiW, Chorus & McKeown, in press; Zessner & Chorus, in press).

Other measures can be applied to water bodies to mitigate cyanotoxin occurrence, including artificial water column mixing, nutrient reduction through sediment removal or treatment, and biomanipulation. Success of these measures is highly dependent on the specific conditions in the water body, as discussed in TCiW, Burch, Brookes & Chorus (in press).

Many reservoir off-take structures (towers) can take water from multiple depths to account for vertical heterogeneity. Variable off-takes enable water layers containing the highest concentrations of cyanobacteria to be avoided. If multiple off-takes are not available (e.g. in small systems), it may be possible, as a temporary measure, to siphon water from a specific depth.

Where conditions allow, the use of bank filtration between source waters and treatment plant inlets can be effective in removing cyanobacteria and in biodegrading dissolved ATXs (TCiW, Brookes et al., in press).

Where possible, sites for recreational activities are best located upwind of bays where scums tend to accumulate.

7.4 Treatment methods and performance

Treatment processes to reduce ATXs in drinking-water are based on two approaches: reducing cell-bound ATXs by physical removal of cells and reducing dissolved ATXs. Although reports of the cell-bound proportion of ATX vary, the majority of ATXs in healthy blooms is likely to be cell bound and therefore effectively removable by physical processes. These include coagulation followed by flocculation, clarification and rapid media filtration, as well as slow sand filtration or membrane filtration.

Filtration processes require care to avoid shear stress that may rupture cells. As cells may lyse in more acidic water, the pH should be kept above 6. Care also needs to be taken to ensure that cyanobacterial and ATX concentrates (e.g. filter backwash, sludges, sludge supernatants) are not allowed to return to the head of the filtration plant during a bloom.

To enhance flocculation, pre-oxidation is sometimes used. Depending on the amounts applied, oxidants can lyse the cells, causing toxin release; at sufficiently high oxidant concentrations, they can also degrade the released toxins (see below). However, elevated cyanotoxin concentrations (including ATXs) typically occur during blooms, which cause a high organic load at the treatment plant. Oxidizing this material without prior filtration is likely to cause high concentrations of disinfection by-products, so filtration before oxidation is recommended.

Dissolved ATXs can be removed by adsorption onto powdered activated carbon (PAC) or granular activated carbon (GAC). Efficacy of removal can be influenced by the type of activated carbon, doses and points of application (PAC), contact times (PAC), flow rates (GAC) and water quality. Biological degradation of ATXs during slow sand filtration and on GAC filters can be very effective, although it may require a lag phase for the degrading bacteria to establish.

ATXs are more resistant to oxidation than other cyanotoxins. Chlorine is not reliably effective, but ozone is effective against ATX. However, the type and concentration of organic substances, as well as pH, strongly affect the amount of disinfectant needed. Elevated organic carbon in bloom situations will substantially increase the disinfectant demand. It is therefore important to validate the disinfectant dose and contact time under the specific conditions of the treatment train and at the point of disinfection. Other oxidants such as chloramine and chlorine dioxide are ineffective against ATXs at doses and contact times normally used in drinking-water treatment.

The treatment methods discussed above can reduce ATX concentrations to well below 30 μg/L. However, validation of efficacy under specific local conditions is important: efficacy is highly

dependent on water quality and other conditions in the treatment system. Validation may include field trials and laboratory investigations such as jar testing. Toxin removal during blooms should be verified by monitoring of ATXs in the finished drinking-water.

After effective treatment, it is important to ensure that drinking-water remains free from cyanobacterial regrowth. This can be accomplished by ensuring that any channels and storages are covered and dark, so that cyanobacteria lack the light necessary for growth. Maintaining chlorine residuals throughout the distribution system will also suppress cyanobacterial regrowth.

For further information, see TCiW, Newcombe, Ho & Capelo-Neto (in press).

8 Conclusions

8.1 Derivation of the provisional health-based reference value

Acute exposure to ATX in animals led to deaths within minutes of gavage administration (Astrachan, Archer & Hilbelink, 1980; Fawell et al., 1999). Since neither of the available repeated toxicity studies identified a nonlethal dose that caused lasting adverse effects, formal guideline values (GVs) (provisional or otherwise) cannot be derived based on the available information. In the 28-day study of Fawell et al. (1999), one of 20 animals in each of two dose groups died without signs that could be attributed to nontreatment effects. If it is conservatively assumed that these animals died from the effects of the toxin, the NOAEL would be 98 µg/kg bw/day, but it could be as high as 2.46 mg/kg bw/day if these two animals were excluded (Fawell et al., 1999). Although GVs cannot be derived due to inadequate data, a "bounding value", or provisional health-based reference value, can be derived for short-term exposure using a highly conservative assumption to define the NOAEL at 98 µg/kg. This value is lower than the estimated NOAEL for exposure via drinking-water calculated from data in Astrachan, Archer & Hilbelink (1980) and the i.p. NOAEL for maternal toxicity identified by Rogers et al., 2005.

There is insufficient information to develop a long-term health-based reference value for ATX.

Default assumptions were applied as described in WHO (2009) for deriving the short-term drinking-water value and WHO (2003) for deriving the recreational water value.

Calculation of provisional short-term drinking-water health-based reference value for ATX:

$$\mathrm{HBRV}_{\mathrm{short-term}} = \frac{\mathrm{NOAEL} * \mathrm{bw} * \mathrm{P}}{\mathrm{UF} * \mathrm{C}} = \frac{98 * 60 * 1.0}{100 * 2}\ \mu\frac{\mathrm{g}}{\mathrm{L}} = 29.4\ \mu\frac{\mathrm{g}}{\mathrm{L}} \approx 30\mu\mathrm{g/L}$$

where

$\mathrm{HBRV}_{\mathrm{short-term}}$ = short-term drinking-water health-based reference value

NOAEL = no-observed-adverse-effect level (98 µg/kg bw/day, based on Fawell et al., 1999)

bw = body weight (default = 60 kg for an adult)

P = fraction of exposure allocated to drinking-water (default for short-term exposure = 100%, considering that drinking-water is expected to be the most likely source of exposure)

UF = uncertainty factor (10 for interspecies variation × 10 for intraspecies variation); an uncertainty factor for database deficiencies was not applied since the NOAEL is lower than the i.p. NOAEL for maternal toxicity

C = daily drinking-water consumption (default = 2 L for an adult).

Calculation of provisional recreational water health-based reference value for ATX:

$$\mathrm{HBRV_{recreation}} = \frac{\mathrm{NOAEL} * \mathrm{bw}}{\mathrm{UF} * \mathrm{C}} = \frac{98 * 15}{100 * 0.25}\ \mu\mathrm{g/L} = 58.8\ \mu\mathrm{g/L} \approx 60\ \mu\mathrm{g/L}$$

where

$\mathrm{HBRV_{recreation}}$ = recreational water health-based reference value

NOAEL = no-observed-adverse-effect level (98 µg/kg bw/day, based on Fawell et al., 1999)

bw = body weight (default = 15 kg for a child)

UF = uncertainty factor (10 for intraspecies variation × 10 for interspecies variation)

C = daily incidental water consumption (default = 250 mL for a child).

8.2 Considerations in applying the provisional health-based reference values

The public should be informed about cyanobacterial blooms in source waters when the water is used for recreation or for producing drinking-water. Cyanobacterial blooms tend to impair the taste and odour of drinking-water even when cyanotoxins are absent, and informing the public about the safety of use of the water is important to avoid people turning to other, less safe sources of water.

For recreational sites with blooms, information and warnings are particularly important. The most common situation is that monitoring cannot occur at sufficiently short time intervals (i.e. daily rather than weekly) to ensure that it captures situations with heavy scums or pronounced greenish turbidity (to the extent that one can barely see one's feet when knee-deep in the water). Site users therefore need information about avoiding scum contact and ingestion of water under such situations. Temporary closure of sites is an option if blooms contain high toxin concentrations, exceeding the recreational water health-based reference value (for further detail, see TCiW, D'Anglada, in press). In determining toxin concentrations that trigger such responses, it is important to consider the actual site of water use (e.g. for raw water abstraction, bathing), since averaged ATX concentrations may underestimate the risk at a particular site. It may also be important to provide information about the possibility that detached aquatic plant-like material, either floating or accumulated in lumps on the beach, can contain high ATX concentrations.

Derivation of the provisional health-based reference values for ATX follows a highly conservative approach. As a result of inadequate data, the provisional health-based reference values derived above do not represent WHO GVs and therefore are not intended for use as scientific points of departure for developing regulations or standards. Nevertheless, a "bounding value" may be useful to guide actions and responses by water suppliers and health authorities. Based on the limited currently available studies of acute and subchronic ATX toxicity, exposure up to the values provided is expected to be safe for adults. Since infants and children can ingest a significantly larger volume of water per body weight (e.g. up to 5 times more drinking-water/kg bw for bottle-fed infants than for adults), it is recommended that alternative water sources, such as bottled water, are provided for bottle-fed infants and small children when ATX concentrations are greater than 6 µg/L for short periods, as a precautionary measure.

The provisional drinking-water health-based reference value is based on a 28-day repeated dose study and so is applicable for short-term exposure. However, because ATX is acutely toxic, it is recommended that any exposure above this value be avoided.

The provisional health-based reference values are based on toxicological data for ATX. It is recommended that, for assessing risk, total ATXs as gravimetric or molar equivalent are evaluated against the health-based reference values, based on a reasonable assumption that HTX has similar toxicity to ATX. There is evidence that dihydro- analogues of ATX and HTX are similarly toxic by the oral route of exposure; hence it would be prudent to include these in determinations of total ATXs, when present.

As described in section 7.2, health-based values can be used within the context of ALFs for early warning and to trigger short-term management responses. For further information on ATX monitoring relative to health-based values in the context of ALFs, see TCiW, Chapter 5 (in press).

References

Aronstam RS, Witkop B (1981). Anatoxin-a interactions with cholinergic synaptic molecules. Proc Natl Acad Sci USA. 78:4639–43.

Astrachan NB, Archer BG, Hilbelink DR (1980). Evaluation of the subacute toxicity and teratogenicity of anatoxin-a. Toxicon. 18(5–6):684–8.

Azevedo, SMFO (in press). Understanding exposure: renal dialysis. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

Bartram J, Correales L, Davison A, Deere D, Drury D, Gordon B, et al. (2009). Water safety plan manual: step-by-step risk management for drinking-water suppliers. Geneva: World Health Organization.

Brookes JD, Burch MD, Grützmacher G, Klitzke S (in press). Managing cyanotoxin risks at the drinking water offtake. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

Burch MD, Brookes JD, Chorus I (in press). Assessing and controlling the risk of cyanobacterial blooms: water-body conditions. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

Campos F, Durán R, Vidal L, Faro LRF, Alfonso M (2006). In vivo effects of the anatoxin-a on striatal dopamine release. Neuroch Res. 31:491–501.

Carmichael W (in press). Human mortality from accidental ingestion of toxic cyanobacteria: a case re-examined. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

Carmichael W, Biggs D, Gorham P (1975). Toxicology and pharmacological action of *Anabaena flos-aquae* toxin. Science. 187(4176):542–4.

Cheng T, Zhao Y, Li X, Lin F, Xu Y, Zhang X, et al. (2007). Computation of octanol–water partition coefficients by guiding an additive model with knowledge. J Chem Inf Model. 47:2140–8.

Chorus I, McKeown RM (in press). Introduction to understanding and managing water use systems. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

Chorus I, Testai E (in press). Understanding exposure: recreation and occupational activities. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

Chorus I, Welker M, editors (in press). Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

D'Anglada L (in press). Public health surveillance, public communication and participation. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

Dietrich DR (in press). Understanding exposure: cyanobacteria as dietary supplements. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

Fawell JK, Mitchell RE, Hill RE, Everett DJ (1999). The toxicity of cyanobacterial toxins in the mouse: II. Anatoxin-a. Hum Exp Toxicol. 18(3):168–73.

Fitzgeorge NLM, Clark SA, Keevil CW (1994). Routes of intoxication. In: Codd GA, Jefferies TM, Keevil CW, Potter E, editors. Detection methods for cyanobacterial (blue–green algal) toxins. First International Symposium on Detection Methods for Cyanobacterial (Blue–Green Algal) Toxins. Cambridge, UK: Royal Society of Chemistry, 69–74.

Health Canada (2018). Guidelines for Canadian drinking water quality: guideline technical document – cyanobacterial toxins. Ottawa: Health Canada.

Heath MW, Wood SA, Barbieri RF, Young RG, Ryan KG (2014). Effects of nitrogen and phosphorus on anatoxin-a, homoanatoxin-a, dihydroanatoxin-a and dihydrohomoanatoxin-a production by *Phormidium autumnale*. Toxicon. 92:179–85.

Hilborn ED, Roberts VA, Backer L, Deconno E, Egan JS, Hyde JB, et al. (2014). Algal bloom-associated disease outbreaks among users of freshwater lakes: United States, 2009–2010. MMWR Morb Mortal Wkly Rep. 63(1):11–15.

Humpage AR, Cunliffe DA (in press). Understanding exposure: drinking water. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

Ibelings BW, Foss A, Chorus I (in press). Understanding exposure: food. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

Ibelings BW, Kurmayer R, Azevedo SMFO, Wood S, Chorus I, Welker M (in press). Understanding cyanobacterial and cyanotoxin occurrence. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

Jarema KA, Poling A, MacPhail RC (2008). Effects of weekly exposure to anatoxin-a and nicotine on operant performance of rats. Neurotoxicol Teratol. 30:220–7.

Kaloudis T (in press). Cyanobacterial taste and odour compounds in water. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

Kofuji P, Aracava Y, Swanson KL, Aronstam RS, Rapoport H, Albuquerque EX (1990). Activation and blockade of the acetylcholine receptor-ion channel by the agonists (+)-anatoxin-a, the N-methyl derivative and the enantiomer. J Pharmacol Exp Ther. 252(2):517–25.

Koskinen AP, Rapoport H (1985). Synthetic and conformational studies on anatoxin-a: a potent acetylcholine agonist. J Med Chem. 28(9):1301–9.

Lawton, L, Metcalf JS, Žegura B, Junek R, Welker M, Török AK, et al. (in press). Analysing cyanotoxins. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

Lilleheil G, Andersen RA, Skulberg OM, Alexander J (1997). Effects of a homoanatoxin-a-containing extract from *Oscillatoria formosa* (cyanophyceae/cyanobacteria) on neuromuscular transmission. Toxicon. 35:1275–89.

Macallan DRE, Lunt GG, Wonnacott S, Swanson KL, Rapoport H, Albuquerque EX (1988). Methyllycaconitine and (+)-anatoxin-a differentiate between nicotinic receptors in vertebrate and invertebrate nervous systems. FEBS Lett. 226:357–63.

MacPhail RC, Farmer JD, Jarema KA (2007). Effects of acute and weekly episodic exposures to anatoxin-a on motor activity in rats: comparison with nicotine. Toxicology. 234:83–9.

Mann S, Cohen M, Chapuis-Hugon F, Pichon V, Mazmouz R, Méjean A, et al. (2012). Synthesis, configuration assignment, and simultaneous quantification by liquid chromatography coupled to tandem mass spectrometry, of dihydroanatoxina and dihydrohomoanatoxin-a together with the parent toxins, in axenic cyanobacterial strains and in environmental samples. Toxicon. 60:1404–14.

Meija J, Coplen TB, Berglund M, Brand WA, De Bièvre P, Gröning M, et al. (2016). Atomic weights of the elements 2013 (IUPAC Technical Report). Pure Appl Chem. 88:265–91.

Méjean A, Dalle K, Paci G, Bouchonnet S, Mann S, Pichon V, et al. (2016) Dihydroanatoxin-a is biosynthesised from proline in *Cylindrospermopsis stagnale* PCC 7417: isotopic incorporation experiments and mass spectrometry analysis. J Nat Prod. 79:1775–82.

Newcombe G, Ho L, Capelo-Neto J (in press). Controlling cyanotoxin occurrence: drinking-water treatment. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

NIST (National Institute of Standards and Technology) (2019). Atomic weights and isotopic compositions with relative atomic masses. Gaithersburg, Maryland: NIST.

Osswald J, Carvalho AP, Guimarães L, Guilhermino L (2013). Toxic effects of pure anatoxin-a on biomarkers of rainbow trout, *Oncorhynchus mykiss*. Toxicon. 70:162–9.

Padisák J, Chorus I, Welker M, Maršálek B, Kurmayer R (in press). Laboratory analyses of cyanobacteria and water chemistry. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

Puddick J, van Ginkel R, Page CD, Murray JS, Greenhough HE, Bowater J, et al. (2021). Acute toxicity of dihydroanatoxin-a from *Microcoleus autumnalis* in comparison to anatoxin-a. Chemosphere. 263:127937.

Puschner B, Hoff B, Tor ER (2008). Diagnosis of anatoxin-a poisoning in dogs from North America. J Vet Diagn Invest. 20:89–92.

Puschner B, Pratt C, Tor ER (2010). Treatment and diagnosis of a dog with fulminant neurological deterioration due to anatoxin-a intoxication. J Vet Emerg Crit Care. 20:518–22.

Rao PL, Bhattacharya R, Gupta N, Parida MM, Bhaskar ASB, Dubey R (2002). Involvement of caspase and reactive oxygen species in cyanobacterial toxin anatoxin-a-induced cytotoxicity and apoptosis in rat thymocytes and Vero cells. Arch Toxicol. 76:227–35.

Rogers EH, Hunter ES, Moser VC, Phillips PM, Herkovits J, Muňoz L, et al. (2005). Potential developmental toxicity of anatoxin-a, a cyanobacterial toxin. J Appl Toxicol. 25(6):527–34.

Shoemaker J, Dietrich W (2017). Single laboratory validated method for determination of cylindrospermopsin and anatoxin-a in ambient water by liquid chromatography/ tandem mass spectrometry (LC/MS/MS). Washington, DC: United States Environmental Protection Agency.

Sieroslawska A (2013). Assessment of the mutagenic potential of cyanobacterial extracts and pure cyanotoxins. Toxicon. 74:76–82.

Skulberg OM, Skulberg R, Charmichael WW, Anderson RA, Matsunaga S, Moore RE (1992). Investigations of a neurotoxic oscillatorialean strain (Cyanophyceae) and its toxin: isolation and characterization of homoanatoxin-a. Environ Toxicol Chem. 11:321–9.

Spivak CE, Witkop B, Albuquerque E (1980). Anatoxin-a: a novel, potent agonist at the nicotinic receptor. Mol Pharmacol. 18:384–94.

Spivak CE, Waters J, Witkop B, Albuquerque EX (1983). Potencies and channel properties induced by semirigid agonists at frog nicotinic acetylcholine receptors. Mol Pharmacol. 23:337–43.

Stevens DK, Krieger RI (1991). Effect of route of exposure and repeated doses on the acute toxicity in mice of the cyanobacterial nicotinic alkaloid anatoxin-a. Toxicon. 29(1):134–8.

Stolerman IP, Alburquerque EX, Garcha HS (1992). Behavioural effects of anatoxin, a potent nicotinic agonist, in rats. Neuropharmacology. 31(3):311–14.

Swanson KL, Allen CN, Aronstam RS, Rapoport H, Albuquerque EX (1986). Molecular mechanisms of the potent and stereospecific nicotinic receptor agonist (+)-anatoxin-a. Mol Pharmacol. 29(3):250–7.

Swanson KL, Aronstam RS, Wonnacott S, Rapoport H, Albuquerque EX (1991). Nicotinic pharmacology of anatoxin analogs. I. Side chain structure–activity relationships at peripheral agonist and noncompetitive antagonist sites. J Pharmacol Exp Ther. 259:377–86.

Testai E (in press). Anatoxins. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

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(5):385–419.

US EPA (United States Environmental Protection Agency) (2015). Health effects support document for the cyanobacterial toxin anatoxin-a. Washington, DC: US EPA (EPA 820R15104).

Valentine WM, Schaeffer DJ, Beasley VR (1991). Electromyographic assessment of the neuromuscular blockade produced in vivo by anatoxin-a in the rat. Toxicon. 29:347–57.

Vidal L, Ballot A, Azevedo SMFO, Padisák J, Welker M (in press). Introduction to cyanobacteria. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.

WHO (World Health Organization) (2003). Guidelines for safe recreational water environments. Volume 1: Coastal and fresh waters. Geneva: WHO.

WHO (World Health Organization) (2009). Policies and procedures used in updating the WHO guidelines for drinking-water quality. Geneva: WHO.

Wonnacott S, Jackman S, Swanson KL, Rapoport H, Albuquerque EX (1991). Nicotinic pharmacology of anatoxin analogs. II. Side chain structure–activity relationships at neuronal nicotinic ligand binding sites. J Pharmacol Exp Ther. 259:387–91.

Wonnacott S, Gallagher T (2006). The chemistry and pharmacology of anatoxin-a and related homotropanes with respect to nicotinic acetylcholine receptors. Mar Drugs. 4(3):228–54.

Wood SA, Puddick J, Fleming R, Heussner AH (2017). Detection of anatoxin-producing *Phormidium* in New Zealand farm pond and an associated dog death. N Z J Bot. 55:36–46.

Yavasoglu A, Karaaslan MA, Uyanikgil Y, Sayim F, Ates U, Yavasoglu NUK (2008). Toxic effects of anatoxin-a on testes and sperm counts of male mice. Exp Toxicol Pathol. 60(4–5):391–6.

Zessner M, Chorus I (in press). Catchment management. In: Chorus I, Welker M, editors. Toxic cyanobacteria in water, second edition. Boca Raton, Florida: CRC Press, on behalf of the World Health Organization, Geneva.