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Cyanobacterial toxins: saxitoxins

Background document for development of WHO *Guidelines for Drinking-water Quality* and *Guidelines for Safe Recreational Water Environments*

World Health Organization

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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.

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

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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; B Lampe, NSF International, United States of America; L Lawton, Robert Gordon University, Scotland; 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
bw	body weight
GTX	gonyautoxin
GV	guideline value
HPLC	high-perfomance liquid chromatography
i.p.	intraperitoneal
i.v.	intravenous
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LWTX	*Lyngbya wollei* toxin
neoSTX	neosaxitoxin
NOAEL	no-observed-adverse-effect level
PSP	paralytic shellfish poisoning
STX	saxitoxin
STXeq	saxitoxin equivalent
STXOL	saxitoxinol
TCiW	*Toxic cyanobacteria in water* (WHO guidebook)
WHO	World Health Organization

Contents

Information on cyanobacterial toxins, including saxitoxins, 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 saxitoxins 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

Saxitoxins (STXs) are naturally occurring alkaloids produced by some marine dinoflagellates and by strains of various species of freshwater cyanobacteria. Marine shellfish are the most likely source of STXs that cause the severe illness known as paralytic shellfish poisoning (PSP). However, these toxins can also be produced by cyanobacteria in drinking-water sources, which means that this route of exposure also needs to be considered and controlled. In most settings, drinking-water is the most likely source of exposure to STXs from fresh waters where surface water is used as the drinking-water source. Limited data suggest that STXs may accumulate in some freshwater food items. Recreational water use may also cause intermittent exposure.

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, filtration and chlorination; if this is not sufficient, ozonation and activated carbon filtration or addition of powdered activated carbon can be effective.

STXs act by blocking sodium channels in nerve cell axons, which inhibits propagation of an action potential along the axon. When this occurs in sensory neurons, symptoms such as tingling and numbness are induced; in motor neurons, muscle weakness or paralysis ensues. The effects of human intoxication by STXs have been well described from numerous cases of PSP. They range from numbness and tingling in the tongue and mouth through muscular weakness in the limbs to, in severe cases, respiratory failure and death. If a person recovers from the acute toxicity, they appear to make a full recovery, and no long-term effects are known.

The guideline values (GVs) for STXs of 3 µg/L in drinking water and 30 µg/L in recreational water are based on a large database of human cases of PSP. These cases involved mixtures of multiple chemical analogues of STX. The cumulative detections of both STX and its structural analogues in a given sample should therefore be evaluated against the GVs.

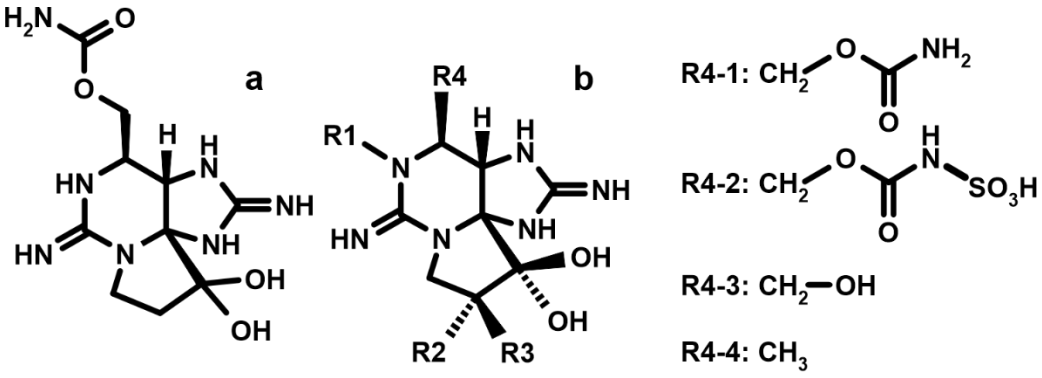
Unlike other cyanotoxins, the drinking-water GV for STXs applies to acute exposure and should not be exceeded even for a short time. The drinking-water GV is derived for bottle-fed infants as the most sensitive subgroup in a population. This is considered appropriate for this cyanotoxin because the GV is for acute exposure, and there is a relatively small margin of safety because a GV based on adults could allow exposure of infants to a concentration of STXs close to the lowest-observed-adverse-effect level. For a 60 kg adult consuming 2 L of drinking-water per day, a 5-fold higher concentration would be tolerable.

1 General description

1.1 Identity

Saxitoxins (STXs), also known as paralytic shellfish poisoning (PSP) toxins, are alkaloids produced by some marine dinoflagellates and various species of cyanobacteria. They were originally found in molluscs after poisonings of humans following consumption of seafood.

While the parent compound was named saxitoxin (STX) after the butter clam (*Saxidermus*) from which it was originally isolated, the term STXs also applies to the family of 57 analogues that includes STX, neosaxitoxin (neoSTX), gonyautoxins (GTXs), C-toxins, decarbamoylsaxitoxins and lyngbyatoxins (LWTXs). Studies often report results as STX equivalents (STXeq). STXeq usually represents the total concentration of STX variants, although in some cases, may represent concentrations adjusted for toxicity (see section 8.2 for more details). The chemical structure consists of a tetrahydropurine group and two guanidine subunits, forming the tricyclic perhydropurine backbone (Fig. 1.1).



R4-1: carbamate toxins, including STX and neSTX; R4-2: *N*-sulfocarbamoyl (or sulfamate) toxins, including GTX-5 and GTX-6; R4-3: decarbamoyl toxins, including decarbamoyl-STX; R4-4: deoxydecarbamoyl toxins, including deoxydecarbamoyl-STX. R1, R2, R3 = H, OH or SO_3H in particular variants. C-toxins have R4-2 and are sulfated at R2 or R3.

Fig. 1.1. (a) Structure of saxitoxin; (b) general structure of saxitoxins and gonyautoxins

1.2 Physicochemical properties

Known physicochemical properties of selected STXs are summarized in Table 1.1. Almost all known STXs are hydrophilic, especially those with one sulfate group. Thus, they are highly soluble in water, with saturation concentrations several orders of magnitude higher than the World Health Organization (WHO) guideline values (GVs) (see section 8.1). The exceptions are STXs produced by *Microsiera* (syn. *Lyngbya*) *wollei* in the freshwater environment, which are characterized by the presence of a hydrophobic side chain with an acetate at C13 (LWTX-1–3, 5, 6) and a carbinol at C12 (LWTX-2, 3, 5) in place of a hydrated ketone. STXs are capable of interconversions, both chemically and enzymatically mediated (see *Toxic cyanobacteria in water* [TCiW], Testai, in press).

Table 1.1. Properties of common saxitoxins in the free base form

Property	Saxitoxin (STX)	Neosaxitoxin (neoSTX)	Decarbamoyl-saxitoxin (dcSTX)	Gonyautoxin 1 (GTX-1)
CASRN	35523-89-8	64296-20-4	58911-04-9	60748-39-2
Chemical formula	$C_{10}H_{17}N_7O_4$	$C_{10}H_{17}N_7O_5$	$C_9H_{16}N_6O_3$	$C_{10}H_{17}N_7O_9S$
Average molecular weight[a] (g/mol)	299.292	315.291	256.266	411.35
Monoisotopic mass[b] (Da)	299.134	315.129	256.128	411.081
K_{ow}[c]	–4.6	–4.3	–4.6	–5.7

CASRN: Chemical Abstracts Service Registry Number

[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 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 or odour, indicating the presence of cyanobacteria in raw water. As this applies only to some strains of some species, the absence of these typical tastes or 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

STXs occur naturally, although high concentrations are typical for water bodies influenced by human activity – for example, by 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 STXs. In marine environments, including brackish water environments, STXs are produced by eukaryotic dinoflagellates of the genera *Alexandrium, Gymnodinium* and *Pyrodinium*. In fresh water, STXs 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*), *Raphidiopsis* (formerly *Cylindrospermopsis*), *Cylindrospermum*, *Aphanizomenon* (some species of which are now classified as *Cuspidothrix* and some as *Chrysosporum*), *Scytonema*, *Lyngbya* (some species of which are now classified as *Microseira* and some as *Moorea*), *Oxynema* (formerly *Phormidium*) and *Planktothrix*. For more information on the new classification of genera, see TCiW, Vidal et al. (in press). For all taxa for which strains producing STXs have been found, nonproducing strains have also been found. Synthesis of STXs is apparently less likely than synthesis of microcystin among strains of *Planktothrix* or *Microcystis*, or synthesis of cylindrospermopsin among strains of *Cuspidothrix* or *Aphanizomenon*.

Production of different congeners seems to be strain specific. Generally no more than three or four congeners are produced by a given strain, with one being dominant. The STX content per cell (i.e. "cell quota") reported for *Dolichospermum circinale* (formerly *Anabaena circinalis*) ranges up to 120 and 450 fg/cell. Higher cell quotas (up to 1300 fg/cell) are reported for a strain of *Scytonema* sp., which, however, has very large cells; thus, in relation to its biomass, with 119 µg/g dry weight, the toxin content of this strain was not exceptionally high.

Reported STX contents in cyanobacterial biomass in field samples range from trace amounts up to 4470 µg/g dry weight. The highest contents have been reported from *Dolichospermum circinale*.

Variations in growth conditions affect the STX content of individual strains only moderately: as with other cyanotoxins, STX cell quotas vary by a maximum of 4-fold with strain-specific responses to environmental factors.

From the data available so far, STXs appear to be mainly confined to viable cells. Where dissolved STXs have been observed, these are thought be primarily released by cell lysis.

The sequence of the STX biosynthesis gene cluster (*sxtA-Z*, approximately 46 kbp) is available for several species: *Raphidiopsis* (formerly *Cylindrospermopsis*) *raciborskii*, *Dolichospermum circinale*, *Aphanizomenon* sp., *Raphidiopisis brookii* and *Lyngbya wollei*. All *sxt* clusters encode biosynthetic enzymes, regulatory genes and transporters. The biosynthetic pathway has been largely elucidated. It involves the incorporation of two arginine residues and steps such as methylation, the addition of an amidino group, and sulfation in respective congeners.

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

2 Environmental levels and human exposure

2.1 Air

STXs are not volatile, and so 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 exposure via this route or on STX concentrations in sprays were found.

2.2 Food

Bioaccumulation of STXs is well documented, primarily for marine shellfish species, most of which are potentially consumed by humans. Far fewer data are available for freshwater species, but a number of studies have demonstrated that bioaccumulation may occur in these organisms to levels of up to several hundred µg/g fresh weight. After transfer to water free from STXs, fish readily eliminate the toxins.

Exposure to STXs through freshwater fish, molluscs and crayfish should be considered for environments where persistent blooms of potentially STX-producing species prevail and such foods are collected for consumption. Molluscs harvested from freshwater environments for human consumption occur in specific localities, and locally specific risk assessments may be relevant for this potential exposure route.

For more information, see TCiW, Ibelings, Foss & Chorus (in press).

2.3 Water

In many settings, the primary waterborne route of human exposure to STXs 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.

STXs have been reported in all types of fresh water, in all continents. However, in the most extensive cyanotoxin surveys, STXs were usually detected in only a small share of the samples and in concentrations <10 µg/L. High concentrations of STXs – up to 193 µg/L – have been reported and may be expected in surface blooms or scums of bloom-forming taxa such as *Raphidiopsis* (previously *Cylindrospermopsis*) and *Dolichospermum* (previously *Anabaena*). The latter species has formed one of the largest reported cyanobacterial blooms, spanning more than 1000 km in the Murray–Darling river system (Australia).

Data on STXs in drinking-water are scarce; if analysis included STX, only trace amounts were found (<0.5 µg/L), suggesting that STXs can be effectively removed by typical drinking-water treatment processes.

Few studies have investigated the chemical breakdown and biodegradation of dissolved STXs. Slow hydrolysis occurs at room temperature, with half-lives for the breakdown reactions in the order of 1–10 weeks, at high pH (10). In natural waters, STX has been shown to persist for up to 2 months.

Recreational activity in surface waters with cyanobacterial blooms may cause exposure to STXs (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 uptake of scum material may potentially cause exposure to high concentrations of STXs. Blooms of most STX-producing cyanobacteria (e.g. *Dolichospermum*) show an intermediate tendency to form surface scums.

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.

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 STXs from fresh waters. However, this assumption is a starting point, and country- or region-specific assessments should take other potential pathways into account. Recreational activities in lakes with cyanobacterial blooms may also expose individuals to high concentrations of STXs, as described in section 2.3. For most situations for the general population, the oral route is the main route of concern.

Patterns and durations of exposure are strongly influenced by region and lifestyle. Estimating total exposure or the relative contribution of particular exposure sources (e.g. food, drinking-water) requires specific analyses of STX 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 STXs, exposure from seafood, especially from marine environments, is likely to be greater than exposure from drinking-water. For most human populations, exposure from food harvested from freshwater environments may be rare and episodic, whereas exposure from drinking-water could occur subchronically over a season. The greatest risk may occur when exposure occurs from food (fresh water and/or marine) and drinking-water together. In these situations, guidelines for STXs in seafood need to be consulted alongside the recommendations in this document.

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).

3 Kinetics and metabolism in humans and laboratory animals

3.1 Absorption

STXs are efficiently absorbed from the gastrointestinal tract, with symptoms occurring minutes to hours after oral exposure to STXs in shellfish. GTX-2/3 crosses the human jejunal epithelium primarily via paracellular diffusion, although it is hypothesized that the toxin causes structural changes in the epithelial tight junction that facilitate uptake in a time- and concentration-dependent manner (Torres et al., 2007). There may be species differences in uptake (Andrinolo et al., 2002a). Absorption also occurs through the mucous membranes of the mouth to produce local numbness within minutes (FAO, 2004).

Absorption in humans was indirectly shown by the detection of STXs at concentrations of 2.8–47 nM by receptor binding assay and high-performance liquid chromatography (HPLC) with fluorescence detection (HPLC-FLD) in the serum of patients who were suffering from acute illness (producing severe hypertension and requiring respiratory support) attributed to four outbreaks of PSP in Alaska in 1994 (Gessner et al., 1997).

In cats given a single oral dose of GTX2/3 at 70 µg/kg, the plasma concentration reached a maximum of 50 ng/mL (125 nM) 150 minutes after dosing. Toxicokinetic modelling indicated that the toxin was freely distributed between the blood and the extravascular space (Andrinolo et al., 2002b).

3.2 Distribution

Specific studies reporting the systemic distribution of STXs are limited to a few animal studies following intravenous (i.v.) or i.p. administration. These studies showed rapid distribution to a range of tissues, including the central nervous system. In Wistar rats given a single i.v. dose of radiolabelled saxitoxinol ([^{3}H]-STXOL), an analogue of STX, radioactivity reached a maximum in most tissues, including brain, 8 hours after dosing (Naseem, 1996). In adult male cats that received a single i.v. injection of STX at 2.7 or 10 µg/kg body weight (bw), the toxin was found in the liver, spleen and central nervous system at low ng/g levels (Andrinolo, Michea & Lagos, 1999).

In rats given single i.p. doses of STX at 5 or 10 µg/kg bw, the toxin was distributed to all parts of the brain. The highest concentration were found in the hippocampus (2.36 pg/mg); slightly lower concentrations were found in the striatum, mid-brain, brain stem and frontal cortex (1.2–1.5 pg/mg); and the lowest concentration was found in the left and right hemispheres (0.8 pg/mg). Approximately 7%, 10% and 18% of the 5 µg/kg bw dose was found in the brain after 30, 60 and 120 minutes, respectively. Following the 10 µg/kg bw dose, 24% was found in the brain after 30 minutes (the only time point examined) (Cervantes Cianca et al., 2007).

3.3 Metabolism

In four Alaskan shellfish poisoning events, the profile of STXs detected in mussels and human biological specimens differed, suggesting that STXs are metabolized in humans. A significant increase in C-1 in comparison with GTX-2 suggested that sulfation of the carbonyl group had

occurred in some patients (Gessner et al., 1997). However, the C-toxin sulfate (at R2/3 in Fig. 1.1b) may also be lost during passage through the acidic conditions of the stomach (Negri, Jones & Hindmarsh, 1995; Aune, 2001), resulting in a bioactivation. These interconversions of sulfated STXs to the more toxic STX and neosaxitoxin (neoSTX) can occur also in shellfish and marine organisms (see Testai et al, 2016). Such interconversions may have significant health consequences, resulting in more severe poisoning episodes than expected if only the toxins present in the water are considered.

In human liver microsomes, STX and GTX-2/3 epimers were found to be N1-oxidized (at R1 in Fig. 1.1b) to neoSTX and GTX-1/4, respectively, and/or glucuronidated on the C12 hydroxyl (García et al., 2009, 2010). N1-oxidation in vivo is supported by evidence that the gastric content of individuals who died after consumption of contaminated shellfish contained only STX and GTX-2/3, while neoSTX and GTX-1/4 were detected in the tissues and urine (García et al., 2004). Hydrolysis of the carbamoyl group of STX was also shown by the presence of decarbamoyl STX in the liver, kidney and lung of these individuals (García et al., 2004). Loss of the sulfate from the sulfocarbomyl of C-toxins can also occur (Humpage, 2008). Glucuronidation will facilitate excretion. However, at least two of these interconversions would result in formation of more potent analogues: loss of sulfate from C-toxins to form carbamate analogues, and N1-oxidation of STX to form neoSTX (Munday et al., 2013; Testai et al., 2016).

In a study in rats using a single i.v. dose of [^{3}H]-STXOL, different percentages of radioactivity were found as unidentified metabolites in various tissues 10 hours after dosing: 19% in the kidneys, 28.5% in the lungs, 41.8% in the heart, 31.8% in the brain and 37.4% in the spinal cord. However, by 48 hours, 75–76% of radiolabel was represented in metabolites. These data may indicate different rates of uptake of hepatic metabolites, compared with the rate of uptake of STXOL, by different tissues, or different rates of metabolism in each of the tissues, or a combination of these (Naseem, 1996). Cats lack some glucuronyl transferases, which helps explain why Andrinolo et al. (1999, 2002b) did not detect metabolites in cats treated with GTX/STX.

3.4 Elimination

In patients recovering from PSP outbreaks in Alaska in 1994, clearance of toxins from serum was evident within 20 hours: STX (65–372 nM) was detected in urine, which was identified as a major route of excretion (Gessner et al., 1997). However, García et al. (2004) detected toxins in the bile as well as the urine, suggesting that faecal excretion may also occur.

In cats (which lack some relevant metabolic pathways – see section 3.3) given a single oral dose of GTX-2/3, 23% of the dose was excreted in the urine after 5 hours, and no toxin was detected in the bile (Andrinolo et al., 2002b). This was also the case when cats were given a single i.v. dose of STX at 2.7 or 10 µg/kg bw: STX was excreted only in urine – within 4 hours, 25% of the administered dose at 2.7 µg/kg and 10% of the administered dose at 10 µg/kg. Renal clearance for the high dose was 0.81 mL/min/kg and for the low dose was 3.99 mL/min/kg (Andrinolo, Michea & Lagos, 1999). The low dose excretion rate is similar to the inulin renal excretion rate in cats, suggesting mainly glomerular excretion at this dose. The authors posited the cause of the low excretion rate for the high dose to be either the severe hypotension that occurred at this rapidly lethal dose and/or a saturable renal excretion mechanism (Andrinolo, Michea & Lagos, 1999).

Rats given a single i.v. dose of [^{3}H]-STXOL had excreted 40% of the dose in urine within 2 hours and 80% after 48 hours. Despite evidence for extensive metabolism of [^{3}H]-STXOL in many tissues, no metabolites were detected in urine (Naseem, 1996).

Rapid excretion in urine was observed after i.v. administration of STX (>96% purity) to rats at a sublethal dose (2 µg/kg). Approximately 19% of the STX dose was excreted in urine within the first 4 hours. At 24 hours, approximately 58% was excreted. At the end of the study period (144 hours), the average total urinary excretion was approximately 68% (Stafford & Hines, 1995).

4 Effects on humans

4.1 Acute toxicity

Fitzgerald, Cunliffe & Burch (1999) reviewed cases of human PSP reported in the literature. They noted a wide range of both lethal and nonlethal reported doses, and identified a symptomatic but nonfatal dose of 124 µg STXeq in a 27-year-old adult female (equivalent to 2.1 µg/kg in a 60 kg person) as the lowest-observed-adverse-effect level (LOAEL). The authors noted similar symptomatic nonlethal doses in a 2-year-old (114 µg) and a 12-year-old (124 µg), and concluded that a single report of 13 µg STXeq causing nonfatal illness in an adult was an outlier.

A review by the Food and Agriculture Organization of the United Nations (FAO, 2004) also found that estimates of the STXeq doses causing either mild effects or death in humans varied considerably, as a result of variations in individual sensitivity, the methods used to determine the dose, and access of victims to competent medical care. Estimates of an oral dose of STXeq causing mild symptoms varied from 120 to 4128 µg, and estimates of a fatal dose were in the range 456–12 400 µg. Although 300 µg STXeq was fatal in some cases, an absence of toxic symptoms has also been reported following consumption of a slightly higher amount of toxin (FAO, 2004).

The FAO (2004) report also noted that children appear to be more sensitive to STX than adults. For example, a 1987 outbreak of PSP was reported with 187 cases and 26 deaths after consumption of clam (*Amphichaena kindermani*) soup. The minimal lethal dose of STXeq was estimated to be about 25 µg/kg bw for a child weighing 25 kg, compared with 86–788 µg/kg bw in four adults who died. Fifty per cent of affected children died, compared with only 7% of adults (Rodrigue et al., 1990; Aune 2001). Prakash, Medcof & Tennant (1971) also noted that the two affected children in their study population became ill from a lower exposure than the average estimated doses of toxin causing adverse effects in adults.

4.1.1 Toxic symptoms

In mild cases of PSP, clinical symptoms include a tingling sensation or numbness around the lips, which usually appears within 30 minutes, gradually spreading to the face and neck. These effects are probably due to local absorption of the STX through the mucous membranes of the mouth. Later, a prickly sensation in the fingertips and toes, headaches, dizziness, nausea, vomiting and diarrhoea usually occur. Sometimes, temporary blindness can occur. Most symptoms are produced within hours but may then last for days. These symptoms precede distinct muscular weakness because sensory nerves, being thinner and having shorter internodes than motor nerves, are always affected first by axonal blocking agents (FAO, 2004).

Moderately severe symptoms associated with higher exposures to STX include numbness of the arms and the legs, which become increasingly weak. The patient may become giddy and have incoherent speech. Cerebellar symptoms such as ataxia, lack of motor coordination and dysmetria are frequent. A tightness around the throat marks the onset of respiratory restriction.

In severe poisoning, the muscular paralysis spreads. The pulse usually remains normal. Death through respiratory paralysis may occur within 2–24 hours of ingestion (FAO, 2004). In patients suffering from PSP during an outbreak in Alaska, a dose-dependent severe hypertension also occurred (Gessner et al., 1997). Because pronounced hypotension is usually seen in animal studies of PSP, as well as some other reports of human poisoning (García et al., 2004), Andrinolo et al. (2002b) hypothesized that hypertension occurs as a central reflex response to an initial hypotension.

If patients survive for 24 hours either with or without mechanical ventilation, the chance of a rapid and full recovery is excellent (FAO, 2004).

4.1.2 Reproductive and developmental effects

Studies designed to assess reproductive or developmental effects of STX exposure were not identified.

4.1.3 Immunological effects

Studies designed to assess immunological effects of STX exposure were not identified.

4.2 Long-term exposure

No studies have been conducted into the effects of long-term exposure of humans to STXs. Prakash, Medcof & Tennant (1971) suggested that fishers regularly exposed to low levels of STXs may develop tolerance.

5 Effects on experimental animals and in vitro systems

5.1 Acute exposure

The acute oral median lethal dose (LD_{50}) of STX in the mouse is 260–263 µg/kg bw, which is about 1/25th the i.p. potency. The acute oral toxicity in eight species varies from 91–100 µg/kg bw in the pigeon to 277–800 µg/kg bw in the monkey (EFSA, 2009). The oral LD_{50} in newborn rats was reported to be 72 µg/kg bw, compared with 531 µg/kg bw in adult rats (Kao, 1966).

In a study using oral dosing (by both gavage and dietary administration) of mice with pure congeners, neoSTX (LD_{50} of 397 µg/kg bw) was found to be more toxic than STX (LD_{50} of 958 µg/kg bw). Administration by gavage gave rise to higher toxicity (i.e. lower LD_{50}). An epimeric mixture of GTX-1/4 had a similar LD_{50} to STX. There were significant differences in the relative potencies of these congeners when administered by the oral and i.p. routes. The oral no-observed-adverse-effect levels (NOAELs) were estimated to be 87, 163, 228, 337 and 486 µg/kg bw for neoSTX, STX, dcSTX, GTX-1/4 and GTX-2/3, respectively (Munday et al., 2013).

The toxicology of STX has been studied in reasonable detail, although most of these studies have used i.p. or i.v. dosing rather than oral dosing (FAO, 2004). The principal effects of a

lethal dose are seen on the respiratory system, heart and skeletal muscles. STX doses of 1–5 µg/kg bw administered i.v. to cats or rabbits cause progressively greater respiratory depression due to paralysis of the muscles of the chest and diaphragm (FAO, 2004). A general weakness of skeletal muscles also occurs. Hypotension is observed in animal studies, although this is rarely seen in human cases (FAO, 2004).

5.2 Short- or long-term exposure

In a study focusing on the safety of neoSTX as a potential local anaesthetic, Zepeda et al. (2014) investigated the toxicity of neoSTX in rats after daily subcutaneous injections of 1, 3 or 6 µg/kg over 12 weeks followed by a 5-week recovery period. These doses were selected as the pharmacologically active dose in a related clinical trial, 3 times this dose and the i.v. LD_{50}, respectively. The LD_{50} values for i.v., intramuscular, subcutaneous and i.p. administrations were 6.06, 11.4, 12.41 and 30.35 µg/kg, respectively. Animals were assessed weekly for behavioural and general morphological changes, as well as consumption of food and drink. At 12 and 17 weeks, five animals from each treatment group were killed, and detailed histology, haematology and blood chemistry were completed. The highest dose (6 µg/kg) induced a significant reduction in weight and food consumption by the end of the treatment period. Changes in serum bilirubin, gamma-glutamyl transferase and aspartate aminotransferase suggested a cholestatic pattern; the authors hypothesized that this may have been due either to effects of neoSTX on the uridine diphosphate-glucuronosyltransferases (known to be involved in neoSTX detoxification) or to the reduced food intake. Effects were no longer seen at the end of the recovery period (Zepeda et al., 2014). The use of i.v., intramuscular, subcutaneous or i.p. administration limits the relevance of these findings for assessing toxicity via the oral route of exposure.

Other studies (e.g. Ramos et al., 2014) used extracts containing STXs. These studies do not provide sufficiently robust results for the derivation of a reference value, since the administered dose cannot be adequately characterized.

5.2.1 Reproductive and developmental toxicity

No reproductive or developmental toxicity studies in mammals were found.

Some evidence of teratogenic activity has been seen in fish and amphibian larvae, in which STX concentrations >10 µg/L caused growth retardation, and 500 µg/L caused malformation and mortality (IPCS, 1984).

5.2.2 Neurological effects

Female Wistar rats were given a culture of the cyanobacterium *Cylindrospermopsis raciborskii* containing STXs in their drinking-water for 30 days. The estimated STXeq concentrations were 3 or 9 µg/L. STXeq doses of approximately 0.8–1.1 µg/kg bw/day in the low-dose group and 2.4–3.4 µg/kg bw/day in the high-dose group can be calculated based on the reported weight range of the rats. After 30 days, the rats were subjected to a range of behavioural tests (the open field habituation test, the elevated plus maze anxiety test, the inhibitory avoidance test and the Morris water maze test). Compared with control rats given nontoxic *Aphanothece* sp. culture and rats receiving STXeq at 3 µg/L, the rats receiving STXeq at 9 µg/L performed significantly worse in the inhibitory avoidance and water maze tests, both of which test memory function (Diehl et al., 2016). Perturbation of antioxidant capacity was observed in the hippocampus and prefrontal cortex of similarly treated rats (Ramos et al., 2014), providing a possible cytotoxicity

mechanism for the effects on memory seen by Diehl et al. (2016), but changes in brain neurotransmitters induced by STXs may also play a role (Cervantes Cianca et al., 2009, 2011).

In rats given a single i.p. dose of STX at 5 or 10 µg/kg bw, dopamine levels increased in various regions of the brain in a time- and dose-dependent manner between 30 and 120 minutes post-dose (Cervantes Cianca et al., 2011). The greatest increase was seen in the brain stem (334% after 120 minutes); increases of 40–80% were seen for the other regions examined. Serotonin levels were also affected (Cervantes Cianca et al., 2009).

One in vitro study examined morphological effects on neurite outgrowth caused by STX (O'Neill, Musgrave & Humpage, 2016a). In human SH-SY5Y and rat PC12 neuronal cells exposed to STX (0.25–3 µg/L) for 7 days, the development of axon-like structures was inhibited in a concentration-dependent manner. In another in vitro assay, the mean relative toxicity of neoSTX was 128 when compared with a United States Food and Drug Administration standard reference material for STX (arbitrarily 100) in the neuroblastoma cell bioassay (Jellett, Stewart & Laycock, 1995). Toxicity was not affected by boiling for 5 minutes in equal volumes of 0.1 or 1.0 N HCl.

5.2.3 Genotoxicity and carcinogenicity

No bacterial or mammalian mutagenicity studies for STX were found.

In cultured human neuroblastoma (Neuro-2A) and monkey kidney (Vero) cells exposed to STX for 24 hours (Melegari et al., 2015), DNA fragmentation was significantly increased relative to controls in both cell lines at all concentrations tested (0.38–3.0 nM; ~0.1–0.9 µg/L). However, the number of micronuclei induced in the cytokinesis-block micronucleus assay was not significantly greater than controls in either cell line.

Oxidative stress is a potential cause of DNA damage; malondialdehyde, a measure of lipid peroxidation, was increased in Neuro-2A cells exposed to STX at a concentration of 3 nM for 24 hours (Melegari et al., 2012). The amount of 5-methyldeoxycytosine as a percentage of total deoxycytosine in Neuro-2A cells was also found to increase when the cells were exposed to STX at 0.75–3 nM for 24 hours (Perreault et al., 2011).

In primary fish neurons treated with a mixture of STXs isolated from *Cylindrospermopsis raciborskii* at an STXeq concentration of 3.0 µg/L, a 15% decrease in cell viability, an increase in markers of oxidative stress, and genotoxicity were observed. An STXeq concentration of 0.3 µg/L had no effect (da Silva et al., 2014).

5.6 Mode of action

The ability of STXs to bind to and block voltage-gated sodium channels in neuronal cells is the most studied action of these toxins (Kao, 1993; Thottumkara, Parsons & Du Bois, 2014; Testai et al., 2016; O'Neill, Musgrave & Humpage, 2016b). Blockade of the sodium channel inhibits propagation of an action potential along neuronal axons, thus reducing or eliminating transmission of a nerve impulse along the axon. When this occurs in sensory neurons, symptoms such as tingling and numbness are induced; when it occurs in motor neurons, muscle weakness or paralysis ensues. STX binds within the pore of the α subunit of the channel, preventing the flow of sodium ions. Ten different isoforms of the human α subunit have been described, each with different distribution, developmental expression and sensitivity to STX (O'Neill, Musgrave & Humpage, 2016b). STX can also block Ca^{2+} and K^{+} channels in cardiac cells, thus preventing the propagation of electrical transmission within the peripheral nerves

and cardiac muscles (Wang, Salata & Bennett, 2003; Su et al., 2004). Although it is clear that most of the clinical effects of STX can be explained by its effects on the sodium channels of peripheral nerves, other symptoms, such as hypertension, may be due to direct effects on the central nervous system, or compensatory reactions to these effects (FAO, 2004), but this has yet to be confirmed.

STX also has inhibitory activity on voltage-gated calcium channels and human ether-a-go-go (hERG) potassium channels. However, the median inhibitory concentration (IC_{50}) values for these effects range from 400- to 1000-fold higher than IC_{50} values associated with inhibition of the sodium channel; thus, the physiological significance of this activity is uncertain (O'Neill, Musgrave & Humpage, 2016b). Oxidative stress may also be involved in some cellular responses to STXs (Ramos et al., 2014; Melegari et al., 2015), although apoptosis of haemocytes in the oyster species *Crassostrea gigas* was shown to be dependent on caspase activation and independent of production of reactive oxygen species (Abi-Khalil et al., 2017).

It has been reported that a number of metazoans have soluble STX-binding proteins that may prevent STX toxicity. An example is saxiphilin, a high-affinity STX-binding protein that is found in a range of species. It appears to have the same toxin recognition strategy in different species, comprising a largely rigid binding site that is similar to the STX-binding site of the voltage-gated sodium channel. This information might be useful in the development of tools for environmental STX monitoring or as an antidote for STX intoxication (Yen et al., 2019).

6 Summary of health effects

6.1 Key studies and key effect(s)

A wealth of evidence from reports of human paralytic poisoning events shows that STXs are acutely toxic. The evidence from a large number of human poisonings by contaminated shellfish indicates that an adult dose of 1.5–2 µg/kg causes diagnostic, but reversible, symptoms of poisoning (Fitzgerald, Cunliffe & Burch, 1999; FAO, 2004; EFSA, 2009). Exposure to a dose high enough to cause severe symptoms is unlikely from ingesting drinking-water, based on the currently available data, although the data for drinking-water are limited (see section 2.3). This is particularly the case where effective water treatment is in place (see section 7.4). Current human evidence suggests that there are no lasting effects in individuals who have suffered mild poisoning (FAO, 2004).

Overall, the quality of the toxicological database for STXs is higher than for other cyanotoxins. However, some key information is still lacking, including pharmacokinetic oral dosing studies in animals (especially in metabolically competent species), comparative oral dosing studies of a range of congeners, and low-dose acute and chronic exposure studies – for example, targeting systemic toxicity, neurodevelopmental effects, and mutagenicity and genotoxicity. Nevertheless, there is strong agreement (FAO, 2004; EFSA, 2009) that the principal mode of lethality is blockade of the voltage-gated sodium channel of peripheral nerves, leading to respiratory paralysis.

Although the point of departure identified for derivation of the guideline value (GV) for STXs is based on human data, the reported human cases vary widely in their estimates of lethal and sublethal doses. This variation reflects uncertainties due to differences in sampling, analytical methods, individuals' sensitivity, and competence of attending medical staff. In contrast, the availability of certified standards for many STX congeners means that animal dosing studies are likely to be more quantitatively reliable than studies of other cyanotoxins.

7 Practical considerations

STXs have been less frequently reported in lakes and reservoirs than other cyanobacterial toxins such as microcystins and cylindrospermopsin. In most countries, blooms producing STXs with health-relevant concentrations of STXs have rarely been reported. Where blooms occur, concentrations of STXs can fluctuate as a result of uneven distribution of blooms in a water body, heterogeneity of clones within blooms and variation in the amount of toxin produced by individual clones. Lethal intoxications of animals (both wild and domestic) have been attributed to STXs. 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 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 STX-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* (formerly *Cylindrospermopsis*) *raciborskii* from one year to the next (TCiW, Ibelings et al., in press).

Monitoring programmes should be adaptive, so that sampling and analysis 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 STX 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 STXs 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 concentrations of STXs.

Wherever possible, toxin analyses should be performed if STXs are suspected. This is because concentrations associated with blooms can vary substantially. This is particularly relevant if cyanobacterial biomass approaches Alert Level 2 for drinking-water because, for some blooms with extremely high STX content, this alert level may not be sufficiently conservative. 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.

7.2 Analytical methods and achievability

Analytical techniques are available for the range of parameters associated with cyanobacterial blooms and STXs. 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 of STX below 3 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) STXs, extraction is performed before analysis. Filtration can be used to separate cells so that intracellular and extracellular fractions can be tested separately. STXs can be extracted from cells with acetic acid, hydrochloric acid or acidified aqueous methanol. A protocol to extract STXs from a range of matrices is available from AOAC International (AOAC Official Method 2005.06). Solid-phase extraction using graphitized carbon or HILIC resins, for example, can be used to concentrate STXs to achieve lower detection limits or for sample clean-up.

As a result of their chemical variability, STXs are one of the most complicated cyanotoxin classes to analyse, requiring care within the laboratory to prevent interconversions. Analysis of STXs of cyanobacterial origin has benefited from advances in analysis of seafood. HPLC methods (e.g. HPLC-FLD) developed for analysis of STXs in the marine environment are suitable for cyanobacterial samples and require prior oxidation. Higher specificity is achieved with various liquid chromatography – mass spectrometry (LC-MS) approaches that are currently used for routine analyses of STXs from marine and freshwater origins. Certified reference material is commercially available for some STX congeners, but the lack of reference material for several STX analogues limits the utility of this method.

Different immunoassays, bioassays, biochemical assays (e.g. saxiphilin enzyme-based assays) and cell-based assays are also available. For immunoassays, the cross-reactivity for individual analogues is considered poor. ELISA (enzyme-linked immunosorbent assay) for STX or neoSTX may have a high sensitivity (for STX alone, a quantification range of 0.02–0.4 µg/L), but cross-reactivity and sensitivity to other variants is poor. ELISAs covering more congeners are under development. A receptor binding assay is available, which provides more reliable results than HPLC, and therefore has been included in the official AOAC methods (AOAC Official Method 2011.27).

Molecular tests have been developed to identify the presence of gene fragments involved in the production of STX 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, assessing compliance with GVs 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 very effective in removing cyanobacteria and, depending on the situation, possibly also in biodegrading dissolved STXs (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 STXs in drinking-water are based on two approaches: reducing cell-bound STXs by physical removal of cells and reducing dissolved STXs. Unless blooms are decaying, a high proportion of STXs 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 concentrates of STXs (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 cells, causing toxin release; at sufficiently high oxidant concentrations, they can also degrade the released toxins (see below). However, elevated cyanotoxin concentrations (including STXs) 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 STXs 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. Whereas biological degradation in slow sand filtration is effective against other cyanotoxins, this has not been established for STXs and may potentially lead to conversion to more toxic analogues.

Oxidation by chlorine or ozone can be effective in degrading dissolved STXs under conditions normally applied for optimal disinfection of drinking-water. However, both the type and concentration of organic substance, 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 STXs.

The treatment methods discussed above can reduce concentrations of STXs below 3 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 STXs 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 guideline value

As STXs are highly potent acutely but there is no indication of chronic toxicity from follow-up of human cases of PSP, a lifetime GV for STXs in drinking-water is not appropriate. Furthermore, the data available do not enable derivation of a lifetime GV. The GV for acute exposure through drinking-water is derived for bottle-fed infants, as the most sensitive subgroup in a population. This is considered appropriate for this cyanotoxin because the GV is for acute exposure, and there is a relatively small margin of safety, as described in section 8.2. All other default assumptions were applied as described in WHO (2009) for deriving the acute drinking-water GV, and WHO (2003) for deriving the recreational water GV.

FAO (2004) identified a LOAEL for mild symptoms of 2.0 µg/kg bw, based on a review of human PSP cases. More recently, EFSA (2009) reviewed about 500 cases of human PSP described in case reports that had estimated the consumption of STXs associated with a range of symptoms (EFSA 2009, Table 16). This analysis identified a LOAEL for STXeq of 1.5 µg/kg bw by assuming an adult body weight of 60 kg. Because many individuals did not show symptoms at much higher estimated intakes, EFSA (2009) reasoned that the LOAEL must be very near the threshold for effects in sensitive individuals. Therefore an uncertainty factor of 3 was applied to the LOAEL "to estimate a NOAEL", establishing an acute reference dose (ARfD) for STXeq of 0.5 µg/kg bw. An uncertainty factor for intraspecies variation was not applied because documented human cases included a wide spectrum of people (occupation, age and sex).

The GVs are derived from data from poisoning events caused by mixtures of STXs, with total STXs expressed as STX concentration equivalents. The GVs therefore apply to total STXs in a sample, not just the parent compound, STX.

These values are supported by data from animal studies: the use of the lowest acute NOAEL for neoSTX of 87 µg/kg bw (Munday et al., 2013) after gavage administration as a point of departure leads to the derivation of an ARfD for neoSTX of 0.87 µg/kg bw (applying an uncertainty factor of 100). This value is of the same order of magnitude as the reference values obtained with human data (Testai et al., 2016).

Calculation of acute drinking-water GV for STXs:

$$\mathrm{GV_{acute}} = \frac{\mathrm{LOAEL} * \mathrm{bw} * \mathrm{P}}{\mathrm{UF} * \mathrm{C}} = \frac{1.5 * 5 * 1.0}{3 * 0.75}\,\mu\mathrm{g/L} = 3.3\ \mu\mathrm{g/L} \approx 3\ \mu\mathrm{g/L}$$

where

GV_{acute} = guideline value for acute exposure

LOAEL = lowest-observed-adverse-effect level (1.5 µg STXeq/kg, based on the human data on PSP reports)

bw = body weight (default = 5 kg for an infant)

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 where surface water is used as the source of drinking-water)

UF = uncertainty factor (3, for use of a LOAEL rather than a NOAEL)

C = daily drinking-water consumption (default = 750 mL for an infant).

Calculation of recreational water GV for STXs:

The calculation is based on a scenario of a child playing in bloom-infested water:

$$\mathrm{GV_{recreation}} = \frac{\mathrm{LOAEL} * \mathrm{bw}}{\mathrm{UF} * \mathrm{C}} = \frac{1.5 * 15}{3 * 0.25}\ \mu\mathrm{g/L} = \ 30\ \mu\mathrm{g/L}$$

where

$GV_{recreation}$ = guideline value for recreational exposure

LOAEL = lowest observed-adverse-effect level (1.5 µg STXeq/kg/day, based on human poisoning data)

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

UF = uncertainty factor (3, for use of a LOAEL rather than a NOAEL)

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

8.2 Considerations in applying the guideline values

The public should be informed about cyanobacterial blooms in source waters when the water is used for recreation or for producing drinking-water. This is particularly important if toxin concentrations in finished drinking-water exceed the GV. As well, 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 in such situations. Temporary closure of sites is an option if blooms contain high toxin concentrations, exceeding the recreational GV (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 STX concentrations may underestimate the risk at a particular site.

As indicated in section 8.1, for assessing risk, the cumulative detection of both STX and its structural analogues should be evaluated against the GVs. This is generally expressed as

STXeq. STXeq can indicate concentration equivalents – calculated by simple addition of the concentrations of all analogues present, each being quantified against an analytical standard for that analogue. This represents a conservative approach to protect human health, in most cases, by assuming that all analogues have comparable characteristics and toxicity to STX. An exception is when the more potent neoSTX is the dominant congener present (see section 5.1). A more precise, usually less conservative approach is to determine STX toxicity equivalents by multiplying the concentration of each analogue by the respective toxicity equivalence factor (TEF) before addition. Where available, oral toxicities should be used in preference to relative i.p. toxicities. Munday et al. (2013) provide the acute oral toxicities of some analogues, and a table of TEFs based on i.p. toxicity in mice has been published by EFSA (2009).

The acute GVs for STXs are based on acute exposure data. A time limit for tolerating concentrations up to 3 µg/L cannot be given because of the lack of data on effects at low doses. Thus, in contrast to other cyanotoxins, short-term and lifetime exposure GVs were not developed, and short-term exceedances of the acute GV should not be permitted. Although there is currently no evidence of health impairments from chronic exposure to low doses of STXs, it is always prudent to implement control measures to reduce the presence of toxic cyanobacterial blooms or their impact on drinking-water supplies as soon as possible (see Chapters 6–10 in TCiW, in press). Limited data show that STX concentrations in drinking-water have almost always been at trace levels (see section 2.3), indicating that conventional water treatment is generally likely to be effective, provided that cell lysis is avoided (see section 7.4).

The drinking-water GV for STXs uses an allocation factor of 100% for drinking-water; however, it may be appropriate to consider reducing the allocation factor for drinking-water in locations with increased risk of coincident water and shellfish exposure (marine or fresh water; see section 2.4). However, it should be noted that GVs for STX in marine shellfish are comparatively high and, in locations where contamination of shellfish is a concern, drinking-water containing STX would contribute a relatively small additional exposure. Nevertheless, it is recommended that health authorities jointly consider and manage such a scenario, particularly given the relatively steep dose–response relationship for these toxins.

For the drinking-water acute GV, the lower body weight and higher likely water intake of infants (as a function of body weight) were used because a GV based on adults could allow exposure of infants to a concentration of STXs close to the LOAEL. For a 60 kg adult consuming 2 L of drinking-water per day, a 5-fold higher concentration than the acute GV would be tolerable.

As described in section 7.1, GVs can be used within the context of ALFs for early warning and to trigger short-term management responses. For further information on STX monitoring relative to GVs in the context of ALFs, see Chapter 5 of TCiW (in press).

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