Cyanobacterial toxins: Saxitoxins

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

Version for Review by Rec Water Group
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Preface

Information on cyanobacterial toxins, including saxitoxins, is comprehensively reviewed in a recent volume to be published by the World Health Organization, “Toxic Cyanobacteria in Water” (TCiW; Chorus & Welker, in press). This covers chemical properties of the toxins and information on the cyanobacteria producing them as well as guidance on assessing the risks of their occurrence, monitoring and management. In contrast, this background document focuses on reviewing the toxicological information available for guideline value derivation and the considerations for deriving the guideline values for saxitoxin in water. Sections 1-3 and 8 are largely summaries of respective chapters in TCiW and references to original studies can be found therein.

To be written by WHO Secretariat
Acknowledgements

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## Abbreviations used in text

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ARfD</td>
<td>Acute Reference Dose</td>
</tr>
<tr>
<td>bw</td>
<td>body weight</td>
</tr>
<tr>
<td>C</td>
<td>Volume of drinking water assumed to be consumed daily by an adult</td>
</tr>
<tr>
<td>GTX</td>
<td>Gonyautoxin</td>
</tr>
<tr>
<td>i.p.</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>i.v.</td>
<td>intravenous</td>
</tr>
<tr>
<td>LOAEL</td>
<td>Lowest Observed Adverse Effect Level</td>
</tr>
<tr>
<td>neoSTX</td>
<td>Neosaxitoxin</td>
</tr>
<tr>
<td>NOAEL</td>
<td>No Observed Adverse Effect Level</td>
</tr>
<tr>
<td>P</td>
<td>Proportion of exposure assumed to be due to drinking water</td>
</tr>
<tr>
<td>PSP</td>
<td>Paralytic Shellfish Poisoning</td>
</tr>
<tr>
<td>PST</td>
<td>paralytic shellfish toxin</td>
</tr>
<tr>
<td>STX</td>
<td>saxitoxin</td>
</tr>
<tr>
<td>STXOL</td>
<td>saxitoxinol</td>
</tr>
<tr>
<td>UF</td>
<td>Uncertainty factor</td>
</tr>
</tbody>
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1.0 EXECUTIVE SUMMARY

2.0 GENERAL DESCRIPTION

2.1 Identity

Saxitoxins (STXs) are natural alkaloids also known as Paralytic Shellfish Poisoning (PSP) toxins (PST). They were originally found in molluscs after poisonings of humans following consumption of seafood.

STXs are a family of 57 analogues, consisting of a tetrahydropurine group and 2 guanidine subunits, representing the tricyclic perhydropurine backbone. For historical reasons, some analogues are named gonyautoxins (GTX) or *Lyngbya wollei* toxins (LWTX).

![Figure 2.1: Structure of saxitoxin (a) and general structure of saxitoxins (STX) and gonyautoxins (GTX). R4-1: carbamate toxins, including STX and neoSTX; R4-2: N-sulfocarbamoyl (or sulfamate) toxins, including GTX5 and GTX6; R4-3 decarbamoyl toxins, including dcSTX. R1, R2, R3=H, OH or SO$_3$H in particular variants.](image)

2.1 Physical and Chemical Properties

All known STXs are hydrophilic, especially those with one sulphate group. Physicochemical properties as far as known are summarized in Table 2.1 for selected saxitoxin analogues.

Table 2.1: Physical and chemical properties of common saxitoxins. N/A: not applicable.

<table>
<thead>
<tr>
<th>Property</th>
<th>saxitoxin</th>
<th>neosaxitoxin</th>
<th>decarbamoyl-saxitoxin</th>
<th>gonyautoxin 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CASRN$^1$</td>
<td>35523-89-8</td>
<td>64296-20-4</td>
<td>58911-04-9</td>
<td>60748-39-2</td>
</tr>
<tr>
<td>Chemical Formula</td>
<td>C$<em>{10}$H$</em>{17}$N$_7$O$_4$</td>
<td>C$<em>{10}$H$</em>{17}$N$_7$O$_5$</td>
<td>C$<em>{9}$H$</em>{16}$N$_6$O$_3$</td>
<td>C$<em>{11}$H$</em>{19}$N$_7$O$_8$S</td>
</tr>
<tr>
<td>Average MW$^2$ (g/mole)</td>
<td>299.292</td>
<td>315.291</td>
<td>256.266</td>
<td>409.380</td>
</tr>
<tr>
<td>Monoisotopic MW (g/mole)</td>
<td>299.134</td>
<td>315.128</td>
<td>256.128</td>
<td>409.102</td>
</tr>
<tr>
<td>Color/Physical State</td>
<td>white powder</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kow$^3$</td>
<td>-4.6</td>
<td>-4.3</td>
<td>-4.6</td>
<td>-5.7</td>
</tr>
<tr>
<td>Solubility in Water</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Solubility in Other Solvents</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$Chemical Abstracts Service Registry Number  
$^2$Molecular Weight  
$^3$logP computation with XLogP3 (Cheng *et al.*, 2007)
2.2 Organoleptic Properties

While none of the known cyanobacterial toxins have been shown to affect the taste or odour of water, some cyanobacterial species produce other compounds such as geosmin and methyl-isoborneol that do affect taste or odour of water, thus indicating the presence of cyanobacteria in raw water. However, as this applies only to some strains of some species, the absence of tastes or odours is not a reliable indicator for the absence of cyanotoxins. Taste or odour thresholds in water are 0.004 ppb for geosmin and 0.006 ppb for methyl-isoborneol (TCiW, Kaloudis, in press).

2.3 Major Uses and Sources

STXs occur naturally, although high concentrations are typical for waterbodies influenced by human activity, i.e. effluents from wastewater or run-off from agricultural land which introduce nutrients that fertilise the growth of phototrophic organisms, including cyanobacteria. There are no known commercial applications for STXs.

In marine environments, including brackish waters, STXs generally are produced by eukaryotic dinoflagellates of the genera *Alexandrium*, *Gymnodinium* and *Pyrodinium* while in freshwater, producers are cyanobacteria. Cyanobacterial taxa for which STX production has been demonstrated belong to Nostocales (*Anabaena circinalis* (Dolichospermum cicinale), *Dolichospermum* lemmernannii, *Cylindrospermopsis raciborskii*, *Aphanizomenon gracile*, *Aph. issatschenkoi*, *Aphanizomenon* sp., *Scytonema* sp.), Oscillatoriales (*Lyngbya wollei*, *Planktothrix* sp., *Oxynema* sp.), and possibly Chroococcales (*Cyanobium* sp.). For all taxa for which strains producing STXs have been found, non-producing strains have also been found. The capacity to synthesize STXs apparently is less frequent than that for microcystin synthesis among strains of *Planktothrix* spp. or *Microcystis* spp.; or for cylindrospermopsin synthesis among strains of *Aphanizomenon* spp.

The production of different congeners seems to be strain-specific with generally no more than three to four congeners co-occurring and with one being dominant. STXs cell quota reported from *Dolichospermum circinale* (published under its former name, i.e. *Anabaena circinalis*) range from 120 to 450 fg/cell. Higher cell quotas up to 1300 fg/cell are reported from *Scytonema* sp. and lower ones of 40 fg/cell for *Aphanizomenon* sp.

Reported toxin contents in cyanobacterial biomass range from a few to up to 4400 µg STXs/g dry weight with highest contents reported from *Dolichospermum circinale*.

Variations in growth conditions affect the STX content of individual strains only moderately: as with other cyanotoxins, STX contents (cell quota) vary only maximally 4-fold with strain-specific responses to individual environmental factors.

STXs are generally confined to viable cells and in cases where dissolved STXs have been observed, these are thought be released primarily due to cell lysis.

The saxitoxin biosynthesis gene cluster (sxA-Z, ca. 46 kbp) sequence is available for several species (*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 like methylation, the addition of an amidino group, the sulfation in respective congeners.
For more details on structural diversity, producing organisms, and biosynthesis see TCiW (Testai, in press).

3.0 ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Air

STXs are not volatile and hence exposure via inhalation would require their dissolution in aerosols. It could also occur through cyanobacterial cells carried in spray, e.g. during storms or in the wake of a power boat. No information on exposure via this route was found nor were data on concentrations in aerosols.

3.2 Food

Bioaccumulation of STXs is well documented primarily for marine shellfish species, most of which are potentially consumed by humans. Much less data are available for freshwater species, but a number of studies have demonstrated that bioaccumulation does occur in these organisms at up to 6.2 mg/g fresh weight. Human consumption of mollusks harvested from freshwater environments is limited to narrow localities, and locally specific risk assessments may be relevant for this potential exposure route.

Fish used for human consumption have also been shown to accumulate STXs, primarily in livers (up to 600 µg STXs/kg fresh weight) and less in muscle tissue (up to 20 µg STXs /kg fresh weight). After transfer to water free of STXs the toxins are readily eliminated. Therefore, exposure to STXs through fish should be considered for environments with persistent blooms of potentially producing species.

For more details see TCiW (Testai, in press).

3.3 Water

In many settings the major water-borne route of human exposure to STX will be the consumption of drinking-water, most likely where it is produced from surface waters with insufficiently effective or non-existent treatment. A further 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 from all continents and all types of freshwaters. In most extensive cyanotoxin surveys, however, STXs were usually detected in only a small share of the samples and only 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 species such as *Cylindrospermopsis raciborskii*, *Dolichospermum lemmermannii*, and *Dolichospermum circinale* (with both species now termed *Dolichospermum* previously published as *Anabaena*). The latter species has formed one of the largest reported cyanobacterial blooms spanning more than 1000 km in the Murray-Darling system (Australia).

Data on STXs in finished drinking-water are scarce and only trace amounts were found (<0.5 µg/L) with a single exception of one sample with 17 µg/L.

Only few studies 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, also at high pH of 10. In natural waters STX has been shown to persist for up to two months.

3.4 Estimated total exposure and relative contribution of drinking-water

Drinking-water is the most likely source of exposure to STX from freshwaters where surface water sources are used. Recreational activities in lakes with cyanobacterial blooms may also expose individuals to STX. Significant dermal or inhalational uptake of CYN during recreational exposure is unlikely, leaving the oral route as the main route of concern.

However, this assumption serves as a starting point: exposure patterns and durations 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 concentrations in respective media in a given setting.

For STX it may be particularly important to include exposure from seafood when assessing risks: this is likely to be greater than exposure from drinking-water, and for most human populations, exposure from food harvested from freshwater environments may be rare and episodic whereas exposure from drinking-water could occur sub-chronically over a season, increasing the chance of co-exposure. Therefore, the greatest risk may occur when exposure occurs from food (both freshwater and marine) and drinking-water together. Where this may be the case, the respective guidelines for STXs in seafood need to be consulted alongside the recommendations in this document.

4 KINETICS AND METABOLISM IN HUMANS AND LABORATORY ANIMALS

4.1 Absorption

STXs are efficiently absorbed from the gastro-intestinal tract, with symptoms occurring minutes to hours after oral exposure to STXs in shellfish. GTX2&3 cross the human jejunal epithelium essentially via paracellular diffusion, although it is hypothesised 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).

In patients from four outbreaks of PSP in Alaska during 1994, PST levels of 2.8-47 nM were detected in the serum during the acute illness, which produced severe hypertension and required respiratory support, and 65-372 nM in urine after acute symptom resolution. The estimated average dose was 9176 µg toxin per person (167 µg/kg) (Gessner et al., 1997).

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

4.2 Distribution
Studies reporting the systemic distribution of STX are limited to a few animal studies following intravenous (i.v.) or intraperitoneal (i.p.) administration that demonstrated rapid distribution to a range of tissues including the central nervous system. In Wistar rats given a single i.v. dose of radiolabeled \(^{3}\text{H}\)-saxitoxinol (STXOL), an analogue of STX, radioactivity reached a maximum in most tissues, including brain, eight hours after dosing (Naseem, 1996). In adult male cats that received a single i.v. injection of 2.7 or 10 µg STX/kg bw, the toxin was found in the liver, spleen and central nervous system at low ng/g levels (Andrinolo et al., 1999).

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

4.3 Metabolism

In four Alaskan shellfish poisoning events, the PST profile differed between mussels and human biological specimens, suggesting human metabolism had occurred. A significant increase in C1 in comparison to GTX2 suggested that sulfation of the carbonyl group had occurred in some patients (Gessner et al., 1997). However, the C-toxin sulfate may also be lost during passage through the acidic conditions of the stomach (Aune, 2001; Negri et al., 1995). In human liver microsomes, STX, neoSTX and GTX3/GTX2 epimers were found to be N1-oxidised and/or glucuronidated on the C12 hydroxyl (Garcia 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 GTX2/3 while neoSTX and GTX1/4 were detected in the tissues and urine (Garcia et al., 2004). Hydrolysis of the carbamoyl group of STX was also shown by the presence of decarbamoylSTX in the liver, kidney and lung of these individuals (Garcia et al., 2004). Cats lack glucuronyl transferases, which helps explain why Andrinolo et al. (1999, 2002b) did not detect metabolites in cats treated with PSP. While glucuronidation will facilitate excretion, at least two of these interconversions (loss of sulfate from C-toxins and N1-oxidation of STX) would be expected to increase toxicity (Munday et al., 2013; Testai et al., 2016).

In a study in rats using a single i.v. dose of radiolabeled \(^{3}\text{H}\)-saxitoxinol, different percentages of radioactivity were found in unidentified metabolites in various tissues 10h after dosing (19% in kidney, 28.5% in the lungs, 41.8% in the heart, 31.8% in the brain and 37.4% in the spinal cord). However, by 48h, 75-76% of radiolabel was represented in metabolites. These data may indicate different rates of uptake of hepatic metabolites, compared to the rate of uptake of saxitoxinol, by different tissues, or different rates of metabolism in each of the tissues, or a combination of these (Naseem, 1996).

4.4 Elimination

In patients recovering from PSP, clearance of toxins from serum was evident within 20 hours and urine was identified as a major route of excretion (Gessner et al., 1997). However, Garcia et al. (2004) detected toxins in the bile as well as the urine, suggesting faecal excretion also occurs.

In cats given a single oral dose of GTX2/3, 23% of the dose was excreted in the urine after 5
hours while no toxin was detected in the bile (Andrinolo et al., 2002b). The clearance rate indicated glomerular filtration as the main mechanism of excretion.

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

Rapid excretion in urine was observed in rats after i.v. administration of radioactively labelled STX at a sub-lethal dose (ca. 2 \(\mu\)g/kg). No radioactivity was detectable in faeces at any time. Four hours after injection, approximately 19\% of the STX dose was excreted in urine. By 24 hours, approximately 58\% of the administered dose was excreted. Average total urinary excretion was approximately 68\% for the full study period, with small quantities of non-metabolized STX still detectable in rat urine up to 144 hours after i.v. administration (Aune, 2001).

In cats given a single i.v. dose of 2.7 or 10 \(\mu\)g STX/kg bw, STX was excreted only by urine; within four hours, 25\% of the administered dose at 2.7 \(\mu\)g/kg and 10\% of the administered dose at 10 \(\mu\)g/kg. Renal clearance at the high dose was 0.81 ml/min/kg and at the low dose 3.99 ml/min/kg. These data suggest that STX excretion mainly involves glomerular filtration (Andrinolo et al., 1999a).

5 EFFECTS ON HUMANS

5.1 Acute toxicity

Fitzgerald et al. (1999) reviewed cases of human PSP reported in the literature to that date. They noted a wide range of both lethal and non-lethal reported doses and identified a non-fatal dose of 124 \(\mu\)g STXeq in a 27-year-old adult female (equivalent to 2.1 \(\mu\)g/kg in a 60kg person) as the LOAEL. The authors noted similar non-lethal doses in a 2-year-old (114 \(\mu\)g) and a 12-year old (124 \(\mu\)g) and concluded that a single report of 13 \(\mu\)g STXeq causing non-fatal illness was an outlier.

A review by FAO (2004) also found that estimates of the STXeq doses causing either mild effects or death in humans varied considerably due to variations in individual sensitivity, the methods used to determine the dose, and access of victims to competent medical care. Estimates of an oral dose causing mild symptoms varied from 120 to 4128 \(\mu\)g STX eq/person while estimates of a fatal dose were in the range 456-12400 \(\mu\)g STX eq/person. While 300 \(\mu\)g STXeq was fatal in some cases, an absence of toxic symptoms had also been reported following consumption of a slightly higher amount of toxin (FAO, 2004).

The FAO (2004) report also noted that children appeared 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 was estimated to be about 25 \(\mu\)g STX eq/kg bw for a child weighing 25 kg compared to 86-788 \(\mu\)g STX eq/kg bw in four adults who died. Fifty percent of affected children died compared to only 7\% of adults (Rodrigue et al., 1990; Aune 2001). Prakash et al. (1971) also noted that the 2 affected children in their study population became ill from less than average estimated doses of toxin.

Toxic symptoms
In mild cases of PSP clinical symptoms include a tingling sensation or numbness around lips, which usually appear within 30 minutes, gradually spreading to the face and neck. These effects
are probably due to local absorption of the PST 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 is also observed. Most symptoms have a quick onset (hours) but may 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).

In moderately severe poisoning, numbness progresses to the arms and the legs, which become increasingly weak. The patient may become giddy and have incoherent speech. Cerebellar manifestations such as ataxia, motor incoordination and dysmetria are frequent. A tightness around the throat marks the onset of respiratory restriction. In severe poisoning, muscular paralysis spreads and becomes deeper. The pulse usually remains normal. Pronounced respiratory difficulty and death through respiratory paralysis may occur within 2 to 24 hours of ingestion (FAO, 2004). In patients suffering from PSP from 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 (Garcia et al., 2004), Andrinolo et al. (2002b) hypothesise that hypertension occurs as a central reflex response to an initial hypotension.

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

5.2 Long-term exposure

No studies have been conducted into the effects of long-term exposure of humans to STXs. Prakash et al. (1971) suggested that fishermen regularly exposed to low levels of STXs may develop tolerance.

5.3 Reproductive and developmental effects

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

5.4 Immunological effects

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

5.5 Mode of action

The acute neurological symptoms seen in humans are consistent with the known action of STXs on the voltage-gated sodium channel in peripheral nerve axons. Blockage of this channel by STX inhibits the flow of sodium ions into the axon, thus reducing or eliminating the action potential that normally transmits a nerve impulse along the axon. When this occurs in sensory neurons, symptoms such as tingling and numbness are induced, and when it occurs in motor neurons muscle weakness or paralysis ensues. Other symptoms, such as hypertension, may be due to direct effects on the central nervous system, or compensatory reactions to these effects, but this has yet to be confirmed.

6  EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO SYSTEMS

6.1 Acute exposure
The acute oral LD$_{50}$ of STX in the mouse is 260-263 µg/kg bw, which is about 1/25$^{th}$ 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 to 531 µg/kg bw in adult rats (Kao, 1966).

In a rare study using oral dosing (by both gavage and dietary administration) of mice with pure congeners, neoSTX was found to be more toxic than STX with dietary LD$_{50}$’s of 397 and 958 µg/kg bw, respectively. Administration by gavage gave rise to higher toxicity (i.e. lower LD$_{50}$’s). An epimeric mixture of GTX1/4 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 NOAELs were estimated to be 87, 163, 228, 337 and 486 µg/kg bw for neoSTX, STX, dcSTX, GTX1/4 and GTX2/3, 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 (FAO, 2004). The principal effects of a lethal dose are seen on the respiratory system, heart and skeletal muscles. Doses of 1-5 µg STX/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).

6.2 Short- or long-term exposure

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. Animals were assessed weekly for behavioural and general morphological changes as well as consumption of food and drink. At 12 and 17 weeks, 5 animals from each treatment group were killed and detailed histology, haematology and blood chemistry completed. The highest dose induced a significant reduction of weight and food consumption by the end of the treatment period. Changes in serum bilirubin, GGT and SGOT suggested a cholestatic pattern which the authors hypothesise may have been due either to neoSTX effects on the uridine diphosphate-glucuronosyltransferases (known to be involved in neoSTX detoxification) or to the reduced food intake. All effects were completely normalized at the end of the recovery period (Zepada et al., 2014).

Other studies are available (e.g. Ramos et al., 2014) in which PSP-containing extracts were used. These studies do not provide sufficiently robust results for the derivation of a reference value, since the administered dose cannot be adequately characterised.

6.3 Reproductive and developmental toxicity

No reproductive or developmental toxicity studies in mammals were found. Some evidence of teratogenic activity has been provided in fish and amphibian larvae, in which STX concentrations >10 µg/L caused growth retardation while 500 µg/L caused malformation and mortality (IPCS, 1984).

6.4 Neurological Effects

Female Wistar rats were given a PST-containing culture of *C. raciborskii* in their drinking-water for 30 days. The estimated toxin concentrations were 3 or 9 µg STX equivalents/L
providing doses of approximately 0.8-1.1 µg STX equivalents/kg bw per day in the low dose group and 2.4-3.4 µg STX equivalents/kg bw per day in the high dose group. 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 to control rats given non-toxic _Aphanothece_ sp. culture and STX low-dose rats, the STX high-dose rats performed significantly worse in the inhibitory avoidance and water maze tests, both of which test memory function (Diehl et al., 2016). Perturbation of anti-oxidant 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 STX-induced changes in brain neurotransmitters may also play a role (Cervantes Cianca et al., 2009, 2011).

In rats given a single i.p. dose of 5 or 10 µg STX/kg bw, dopamine levels increased in various regions of the brain in a time- and dose-dependent manner between 30 and 120 min post-dose (Cervantes Cianca et al., 2011). The greatest increase was seen in the brain stem (334% after 120 min), with increases of between 40% and 80% 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 STXs (O’Neill et al., 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 to a USFDA standard reference material for STX (arbitrarily 100) in the neuroblastoma cell bioassay (Jellet et al., 1995). Toxicity was not affected by boiling for 5 min in equal volumes of either 0.1 or 1.0 N HCl.

### 6.5 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 h (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 and malondialdehyde, a measure of lipid peroxidation, was increased in Neuro-2A cells exposed to 3 nM STX for 24 h (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 0.75 – 3 nM STX for 24 h (Perreault et al., 2011). In primary fish neurons treated with a mixture of STXs isolated from _C. raciborskii_ at 3.0 µg STX eq/L, a 15% decrease in cell viability, increase in markers of oxidative stress, and genotoxicity was observed. A concentration of 0.3 µg STX eq/L had no effect (da Silva et al., 2014).

### 6.6 Mode of action

The ability of STXs to bind to and block voltage-gated sodium channels is the most studied action of these toxins (O’Neill et al., 2016b). Blockade of the sodium channel inhibits propagation of an action potential along neuronal axons, limiting transmission of nerve impulses to muscles. 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 distributions, developmental expression and sensitivity to STX (O’Neill et al., 2016b). Whilst it is clear that most of the clinical effects of STX can be explained by its effects on the sodium channels of peripheral nerves, a potential role for central nervous system actions remains possible (FAO, 2004). STX also has inhibitory activity on voltage-gated calcium channels and human ether-a-go-go (hERG) potassium channels but the IC₅₀’s are 400-1,000-fold higher than for the sodium channel and so the physiological significance of this activity is uncertain (O’Neill et al., 2016b). Oxidative stress may also be involved in some cellular responses to STXs (Ramos et al., 2014; Melegari et al., 2015).

7 SUMMARY OF HEALTH EFFECTS

7.1 Key studies and Key effect(s)

There is a wealth of evidence from reports of human paralytic poisoning events that STXs are acutely toxic. The evidence from a large number of human poisonings from contaminated shellfish indicate that an adult dose of 1.5-2 µg/kg causes diagnostic, but reversible, symptoms of poisoning (Fitzgerald et al., 1999; FAO, 2004; EFSA, 2009). Exposures sufficient to cause severe symptoms are unlikely to occur from ingesting contaminated water. 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 STX is higher than for other cyanotoxins. However, some key information is still lacking, including of pharmacokinetic oral dosing studies in animals, particularly in metabolically competent species, comparative oral dosing studies of a range of congeners, low dose acute and chronic exposure studies targeting e.g., neurodevelopmental effects, and mutagenicity and genotoxicity. Nevertheless, there is strong agreement that the principal mode of lethality is blockade of the voltage-gated sodium channel of peripheral nerves leading to respiratory paralysis.

Although the PoD identified for derivation of the guideline value for STX is based on human data, the reported human cases vary widely in their estimates of lethal and sub-lethal doses reflecting uncertainties due to sampling, analytical methods, interindividual variation in sensitivity, and competence of attending medical staff. In contrast, the availability of certified standards for many STX congeners has meant that animal dosing studies are likely to be more quantitatively reliable than studies of other cyanotoxins.

8 PRACTICAL CONSIDERATIONS

Saxitoxins occur less frequently in lakes and reservoirs than other cyanobacterial toxins like microcystins and cylindrospermopsin, and in most countries STX-producing blooms with health-relevant concentrations of STX are rarely reported. Where blooms occur STX concentrations can fluctuate due to 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 animal intoxications (of wild as well as of domestic animals) 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 give guidance on multiple barriers against cyanotoxins in water including controlling nutrient loads from the catchment, managing water bodies, optimizing sites for drinking-water offtakes 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 barriers used to mitigate cyanotoxin risks through developing a water safety plan (TCiW; Chorus & McKeown, in press).

8.1 Source control

For planktonic toxic cyanobacteria the prevention of blooms in source waters is the key to long-term control of the risks they represent. The most sustainable approach to achieve this is to keep concentrations of plant nutrients low. Most cyanobacteria typically proliferate under eutrophic conditions i.e., at elevated concentrations of nutrients, in particular of phosphorus, and total phosphorus concentrations below 20-50 µg/L will limit the development of cyanobacterial blooms in most situations (TCiW; Chorus & McKeown, in press; Zessner & Chorus, in press). A number of measures within water bodies can mitigate cyanotoxin occurrence, including e.g. artificial water column mixing, nutrient reduction through sediment removal or treatment, or biomanipulation. Their success is highly dependent on the specific conditions in the water body, as discussed in TCiW (Burch et al., in press).

Many reservoir off-take structures (towers) can take water from multiple depths to account for vertical heterogeneity. Variable offtakes enable avoiding water layers containing the highest concentrations of cyanobacteria. 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 both for removing cyanobacteria and for biodegradation of dissolved MC (TCiW; Brookes et al., in press). Where possible, sites for recreational activities are best located upwind of bays where scums tend to accumulate.

8.2 Monitoring

While cyanobacteria can be present in surface waters at low numbers throughout the year the occurrence of blooms producing significant concentrations of toxins tend to be short-lived and often seasonal events. Monitoring of source waters should include surveillance for factors that can support the growth of cyanobacteria including total phosphorus, temperature, water residence time, pH and Secchi disc transparency (for detail see TCiW; Padisák et al., in press). On site visual assessment of turbidity with greenish discolouration or scums and microscopy are effective low cost direct methods that can trigger increased vigilance if STX-producing cyanobacteria are observed. In many cases monitoring over several seasons can establish the likely occurrence and timing of favourable conditions for cyanobacterial growth as well as the taxonomic composition and magnitude of blooms in individual water bodies. For example, a lake with regular seasonal blooms of *Aphanizomenon* in late summer is unlikely to shift to perennial blooms of *Cylindrospermopsis* from one year to the next (TCiW; Ibelings et al., in press).

Monitoring programmes should be adaptive with sampling and testing being increased when there is evidence of increasing cell numbers. An Alert Level Frameworks (ALF) have been described both for drinking-water and for recreational water use. These include various criteria to trigger particular analyses and risk mitigation measures (TCiW; Humpage et al., in press; Chorus & Testai, in press). As described in the ALFs, 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 discoloration, and these may be overlooked. Therefore, if the presence of cyanobacteria is suspected, microscopic examination for the presence of potentially STX producing cyanobacteria is important. As
blooms develop monitoring can be expanded to include quantitative measures of cyanobacterial biomass indicating potential toxin concentrations such as cyanobacterial biovolumes or chlorophyll-α, or direct analyses of STX concentrations. Wherever possible, toxin testing should be performed as concentrations associated with cyanobacterial blooms can vary substantially. Toxin data may well allow avoiding or lifting restrictions of site use where these were based on biovolume or chlorophyll-α concentrations.

### 8.3 Analytical methods and achievability

Analytical techniques are available for the range of parameters associated with cyanobacterial blooms and associated STX. The complexity, expertise requirements and costs of monitoring increase from relatively simple visual inspections to testing for phosphorus, pH, Secchi disc transparency, cell numbers, species identification, biovolumes and chlorophyll-α determination. Testing for STX using liquid-chromatography-mass spectrometry (LC/MS) or high-performance liquid chromatography mass spectrometry (HPLC) is the most complex and time consuming.

For cell-bound STXs, an extraction step is performed prior to analysis. Due to their chemical variability, STXs are one of the most complicated cyanotoxin classes to measure. Analysis of STXs of cyanobacterial origin has benefitted from advances in PST analysis in seafood. HPLC methods developed for the analysis of STXs in the marine environment are suitable for cyanobacterial samples but the lack of reference material for several STX analogues limits the performance. A higher specificity is achieved with various LC-MS approaches that are currently applied for routine analyses of STXs from marine and freshwater origins. For some STX congeners certified reference material is commercially available.

Immunoassays, biochemical assays (e.g. Saxiphilin enzyme-based) are also available. For immunoassays, antibodies against saxitoxin or neosaxitoxin have been produced but the cross-reactivity for individual analogues is considered poor. ELISA kits specifically for saxitoxin alone have a quantification range from 0.02 – 0.4 µg/L and thus are sufficiently sensitive for analyzing whether or not water meets the guideline levels. However, the response for the other STXs is highly variable. If the dominant STXs are known, then it may be possible to calibrate the assay to these analytes. ELISA kits are also available directed to neo-saxitoxin, and these also demonstrate high sensitivity (0.03 – 0.1 µg/L) although their sensitivity to saxitoxin and the other variants is low (for more detail see TCiW; Metcalf et al., in press).

While these methods were developed for the analysis of water samples, applying them to more complex matrices such as food or stomach contents requires prior clean-up.

### 8.4 Treatment methods and performance

Treatment processes to reduce STX in drinking water are based on two approaches; reducing cell-bound STX by physical removal of the cells and reducing dissolved STX (TCiW; Newcombe et al., in press). In healthy blooms a high proportion of STX are cell bound and therefore effectively removable by physical processes, i.e. coagulation followed by flocculation, clarification and rapid media filtration as well as by slow sand filtration or membrane filtration. Care needs to be taken to avoid or minimise pre-filtration treatments such as chlorination before filtration as this causes cell lysis and release of STX. Further, 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 STX concentrates (e.g. filter backwash, sludges and sludge supernatants) are not allowed to return to the head of filtration plants during a bloom.
Dissolved STX can be removed by adsorption onto powdered or granular activated carbon (PAC or 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. While biological degradation in slow sand filtration is effective against other cyanotoxins, this has not been established for STX and may even lead to conversion to more toxic analogues.

Oxidation by chlorine or ozone can be effective for destruction of dissolved STX under conditions normally applied for optimal disinfection of drinking-water. However, other oxidants such as chloramine and chlorine dioxide have been shown to be ineffective oxidants against STX. Ozone or chlorine can be used to reduce concentrations of STX prior to filtration. Also, where removal of cells by filtration is inadequate or absent, if applied in sufficient amounts these oxidants can both lyse the cells and destroy the released toxins. However, elevated cyanotoxin concentrations typically occur during blooms which cause a high organic load to the treatment plant. As oxidising this without prior filtration is likely to cause high concentrations of disinfection byproducts, filtration prior to oxidation is recommended.

The treatment methods discussed above are able to reduce STX concentrations below the Guideline value of 3 µg/L. However, validation of efficacy under specific local conditions is important, this applies in particular to slow sand filtration and chlorination: their efficacy is highly dependent on the specific water quality and further conditions in the treatment system. Validation may include field trials and laboratory investigations such as jar testing. Verification of removal during blooms should be undertaken by monitoring STX in finished drinking-water.

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

9 CONCLUSIONS

9.1 Derivation of the guideline-value

As STXs are highly potent acutely but there is no indication of chronic toxicity, a lifetime guideline value for STX in drinking-water is not appropriate. The guideline value for acute exposure through drinking-water is derived for bottle-fed infants as the most sensitive subgroup in a population. For a 60 kg adult consuming 2 L of drinking-water per day, a 5-fold higher concentration would be tolerable.

FAO (2004) reviewed the 500 reported cases of human paralytic shellfish poisoning and a provisional LOAEL of 2.0 µg STXeq/kg bw was established. A 3x UF applied to the LOAEL resulted in an Acute Reference Dose (ARfD) of 0.7 µg STX eq/kg bw. Based on the same data, EFSA (2009) identified a LOAEL of 1.5 µg STXeq/kg bw and applied a 3x UF, establishing an ARfD of 0.5 µg STX eq/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) and mild illness is readily reversible.

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

**Calculation of acute drinking-water guideline value for saxitoxins:**

\[
GV_{\text{acute}} = \frac{\text{LOAEL} \times \text{bw} \times \text{AF}}{\text{UF} \times \text{C}} = \frac{1.5 \times 5 \times 1.0}{3 \times 0.75} \mu\text{g/L} = 3.3 \mu\text{g/L} \approx 3 \mu\text{g/L}
\]

AF: proportion of exposure assumed to be due to drinking water, 1.0;
bw: body weight (WHO standard is 5 kg for an infant);
C: daily water consumption (0.75 L per day for an infant);
LOAEL: lowest observed adverse effect level, (1.5 µg/kg STX equivalents based on the human data on paralytic shellfish poisoning reports);
UF: uncertainty factor (3 for LOAEL to NOAEL).

**Calculation of recreational water guideline value for saxitoxin:**

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

\[
GV_{\text{rec}} = \frac{\text{LOAEL} \times \text{bw}}{\text{UF} \times \text{C}} = \frac{1.5 \times 15}{3 \times 0.25} \mu\text{g/L} = 30 \mu\text{g/L}
\]

bw: body weight (assumed to be 15 kg for a child);
C: incidental water intake assumed to be 0.25 L during primary contact (e.g., energetic play, swimming, falling out of a boat);
LOAEL: lowest observed-adverse-effect level (1.5 µg/kg per day, based on human poisoning data);
UF: uncertainty factor (3, for converting LOAEL to NOAEL).

**9.2 Considerations in applying the guideline values**

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. Site users therefore need information about avoiding scum contact and ingestion as well as situations with pronounced greenish turbidity, i.e. to the extent that one cannot see one’s feet when knee-deep into the water. Temporary closure of sites is an option if blooms contain high toxin concentrations, exceeding the recreational guideline values (for further detail see TCiW, d’Anglada et al.).

For the application of these guidance values, the total concentration of STX variants is generally expressed as STX concentration equivalents. This would represent a conservative approach to protect human health, when the most toxic variant is set as the reference unit. By applying the TEF approach, as EFSA (2009) did, based on the mouse bioassay with i.p. injection, STX was the reference variant. After the adoption of the EFSA Opinion it has been shown that the TEF values based on mouse bioassay are different from those calculated using the oral LD$_{50}$, according to which neoSTX would have a TEF>1, being more toxic than STX. However, if the LD$_{50}$ for neoSTX is used as PoD a similar reference value was obtained, supporting the guideline value presented here.

The acute guideline values for STXs are based on acute exposure data. A time limit for
tolerating concentrations up to 3 µg/L cannot be given due to the lack of data on effects at low doses. Thus, in contrast to other cyanotoxins, short-term and lifetime exposure guideline values were not developed, and short-term exceedances of the acute guideline value should not be permitted. While concentrations observed in drinking-water so far have almost always been below 10 µg/L (see section 3.3 above) and there is no evidence of health impairments from chronic exposure to low doses of STX, 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).

The drinking-water guideline values for STX uses an allocation factor of 100%; however, a lower allocation factor for drinking-water can be used in locations with increased risk of coincident water and seafood exposure causing severe adverse effects.

The recreational guideline value is based on exposure of a child because the lower body weight and higher likely water intake (as a function of body weight) was considered worst case (WHO, 2003). Further, for the drinking-water acute guideline value, the lower body weight and higher likely water intake (as a function of body weight) was used because a guideline value based on adults could allow exposure of infants to a PST concentration close to the LOAEL.

10 REFERENCES (still need some tidying up)


Cervantes Cianca CR, Faro LF, Durán BR, Alfonso PM. (2011) Alterations of 3,4-


