OPCW **Scientific Advisory Board**

Twenty-First Session
23 – 27 June 2014

SAB-21/WP.4
28 February 2014
ENGLISH only

SAXITOXIN FACT SHEET

1. Challenges in development of analytical methods for saxitoxin have been considered by the Scientific Advisory Board since its Sixth Session, in particular through the Temporary Working Group on Sampling and Analysis (which held its final meeting in September 2012). In addition to being listed in Schedule 1 of the Chemical Weapons Convention, saxitoxin is covered by the Biological and Toxins Weapons Convention. Furthermore, in its naturally occurring form saxitoxin is a cause of paralytic shellfish poisoning; saxitoxin is also used in diagnostic medicine.

2. A first version of a fact sheet on saxitoxin was developed in 2011 and made available in the public domain (in Annex 5 of SAB-17/1, dated 23 November 2011). A revised version was issued in 2012 (in Annex 4 of SAB-18/1, dated 19 April 2012).

3. For ease of reference by States Parties, the fact sheet (as published in 2012) is hereby issued under separate cover.

Annex:

Saxitoxin Fact Sheet

CS-2014-8414(E) distributed 03/03/2014

Annex

SAXITOXIN FACT SHEET

1. Introduction

1.1 Saxitoxin (STX) is a neurotoxin which is naturally produced by certain species of marine dinoflagellates (including Alexandrium sp., Gymnodinium sp., Pyrodinium sp.) and cyanobacteria (including Anabaena sp., some Aphanizomenon spp., Cylindrospermopsis sp., Lyngbya sp., Planktothrix sp.). Ingestion of saxitoxin, usually through shellfish contaminated by toxic algal blooms, is responsible for the human illness known as paralytic shellfish poisoning (PSP).

1.2 The term saxitoxin has also been used generically to refer to structurally related neurotoxins (analogues of saxitoxin) produced by the same microorganisms. These include neosaxitoxin (neoSTX), the gonyautoxins (GTX) and decarbamoylsaxitoxin (dcSTX). These molecules range in molecular mass from 250 to 500 Da, depending on the substituent groups.

2. Nomenclature

2.1 The term saxitoxin originates from the species name of the butter clam (Saxidomus giganteus) from which the toxin was first isolated.

2.2 A survey of the literature demonstrates how the nomenclature of saxitoxin has changed since the toxin was first isolated in 1957.[1] The term 'saxitoxin' was originally used in reference to the dihydrochloride salt.[2] In the early 1980s, one chemistry manual referred to the free base as saxitoxin.[3] Since the late 1980s, the di-cation, in which the two basic nitrogens are protonated, has frequently been referred to as saxitoxin, without specifying the associated anions (i.e. which salt).[4] More recently (and since the negotiations on the Chemical Weapons Convention were concluded in 1992), the nomenclature of saxitoxin has become more specific, distinctions are now made between saxitoxin hydrate[5] (free base) and the dihydrochloride salt, which have different CAS numbers.[6] To avoid confusion, the former is hereinafter referred to as saxitoxin hydrate (free base). For consistency, the dihydrochloride salt should strictly be referred to as saxitoxin hydrate dihydrochloride salt.

---

[1] R. J. Mathews, 'Saxitoxin and the CWC: Personal Recollections and Reflections', Presentation to the Thirteenth Session of the Scientific Advisory Board, Annex 4 in Report of the Thirteenth Session of the Scientific Advisory Board, SAB-13/1 (1 April 2009).

[2] See, for example, Dictionary of Organic Compounds, 4th Edition (1965); SIPRI, The Problem of Chemical and Biological Warfare Vol. I, pp. 67-68, (1971), P.J. Scheuer, Chemistry of Marine Natural Products, (1973).

[3] Dictionary of Organic Compounds, 5th Edition (1982).

[4] See for example, The Concise Encyclopedia Biochemistry, 2nd Edition (1988), The Merck Index 11th Edition (1989); The Merck Index 14th Edition (2006).

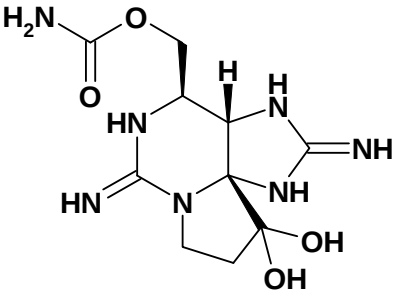
[5] The inclusion of 'hydrate' in the name arose from initial uncertainty on whether the 10 position on the molecule was a ketone [C=O] or its hydrate [$C(OH)_2$].

[6] Richard J. Sax Sr, Sax's Dangerous Properties of Industrial Materials, 9th Edition (1995); saxitoxin hydrate CAS No 35523-89-8; saxitoxin dihydrochloride CAS No 35554-08-06.

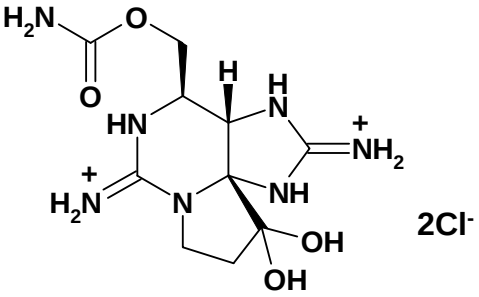
2.3 The systematic IUPAC name for naturally occurring (+)-saxitoxin hydrate (free base) is: (3aS, 4R, 10aS) 2,6-diamino-4-[[(aminocarbonyl)oxy]methyl]-3a,4,8,9-tetrahydro-1H,10H-pyrrolo[1,2-c]purine-10,10-diol.

2.4 Saxitoxin is traded, e.g. in diagnostic kits, mainly as the dihydrochloride salt or as the diacetate salt. Saxitoxin hydrate (free base) has poor stability and is rarely traded.

3. Structure of saxitoxin



saxitoxin hydrate (free base)



saxitoxin dihydrochloride salt

4. Sources of saxitoxin

4.1 Saxitoxin can be isolated (usually as a salt) from bivalve molluscs (e.g. the butter clam Saxidomas giganteus) that have accumulated PSP-producing dinoflagellates (e.g. Gonyaulax catanella) during feeding. In one reported experiment about 8 tonnes of clams were processed to produce a single gram of saxitoxin.[7]

4.2 Saxitoxin can also be produced by liquid culture of the dinoflagellate species Gonyaulax catanella, although yields have been well below those that would be required to use saxitoxin as a chemical warfare agent.

4.3 Saxitoxin has been synthesised in very small quantities and with considerable difficulty. Saxitoxin was first synthesised in 1977 in a 17-step synthesis with an overall yield of 0.2%.[8] More recently, saxitoxin has been synthesised in a 19-step synthesis with an overall yield of 1.6%.[9]

---

[7] WHO, Public health response to biological and chemical weapons, (World Health Organization, Geneva, 2004).

[8] H. Tanino, T. Nakata, T Kanedo and Y. Kishi, A Stereospecific Total Synthesis of d,l-Saxitoxin, *J. Amer. Chem. Soc*., 1977, 2818.

[9] J. Fleming and J. Du Bois, Total Synthesis of (+) Saxitoxin, *J. Amer. Chem. Soc*., 2006, 3926.

5. Main clinical features[10]

Saxitoxin is a powerful neurotoxin that binds with high affinity to sodium channels on cell membranes, inhibiting the influx of sodium ions into cells, with resulting suppression of cell action potentials, leading to muscle paralysis.[11] Following ingestion of saxitoxin, the onset of symptoms is typically within 10 - 60 minutes. Numbness or tingling of the lips and tongue (attributable to local absorption) spreads to the face and neck, followed by a prickling feeling in fingers and toes. With moderate to severe exposure, the paralysis spreads to the arms and legs. Motor activity is reduced, speech becomes incoherent and respiration laboured, with death from respiratory arrest. The terminal stages may occur within 2 – 12 hours. Fatalities in adults have been reported following ingestion of 0.5 – 12.4 mg. Following exposure through inhalation, most of the symptoms would occur much faster.

6. Protective Measures[12]

6.1 Diagnosis of saxitoxin poisoning is confirmed by detection of the toxin, most commonly using enzyme linked immunosorbent assay (ELISA), other immunoassay, LC-fluoresence or LC-MS/MS in samples of, for example, stomach contents, water or food. A mouse bioassay, commonly used in the past, is declining in use.

6.2 No specific antidotes to saxitoxin poisoning exist, and treatment is symptomatic. The toxin is normally cleared rapidly from the body via the urine, so that casualties who survive for 12 – 24 hours usually recover. Diuretics may help. Specific antitoxin therapy has been successful in animals. No vaccine against saxitoxin exposure has been developed for human use.

7. Saxitoxin: Peaceful Applications

Saxitoxin is a component in diagnostic testing kits for PSP. It is also used as a tool in neurochemical research, including electrophysiological studies.

8. Saxitoxin as a CB Weapon

8.1 Saxitoxin hydrate dihydrochloride salt was first isolated in small quantity at the US Army Fort Detrick laboratory in the 1950s, designated as Agent TZ, and was

---

[10] WHO, Public health response to biological and chemical weapons, (World Health Organization, Geneva, 2004).

[11] The dication is the form of saxitoxin that binds to the sodium channels on cell membranes. At physiological pH saxitoxin exists predominantly as the di-cation, partly as a mono-cation.

[12] WHO, Public health response to biological and chemical weapons, (World Health Organization, Geneva, 2004).

investigated as a potential weapon.[13] Agent TZ was apparently weaponised in the M1 Biodart (E1) flechette system in the 1950s and 1960s.[14]

8.2 Saxitoxin hydrate (free base) and its salts are soluble in water. The free base dissolves in water to produce an alkaline solution but is unstable at this pH. Saxitoxin has good stability in water at neutral and weak to moderately acidic pH. It is supplied in acidic solution in diagnostic kits. Dispersal of saxitoxin hydrate dihydrochloride as an aerosol is feasible. No cases of human inhalation exposure have been reported in the medical literature, but animal experiments suggest that the entire syndrome is compressed, and that death may occur within minutes.[15]

9. Saxitoxin and the CWC

Saxitoxin was proposed for inclusion in the CWC Schedules of Chemicals by the USA in 1984,[16] and was subsequently included in the CWC Rolling Texts within Schedule 1, with a footnote reflecting the view of some negotiators that saxitoxin would be more appropriate in Schedule 2. From the record of negotiations it appears that what negotiators wanted to include in the Schedules was the form of saxitoxin that had been weaponised in the past (i.e. Agent TZ, the dihydrochloride salt), and other forms of weaponisable saxitoxin.[17] When CAS Numbers were assigned to the chemicals in the CWC Rolling Text in the late 1980s, saxitoxin was assigned the CAS Number of saxitoxin hydrate (free base) on the understanding that the CAS Numbers were intended to be 'identification aids' rather than 'unique identifiers' for the various scheduled chemicals.[18] In the CWC 'end-game' in 1992, it was agreed that 'saxitoxin' would be placed in Schedule 1.

10. Verification

The identification of saxitoxin in environmental and man-made samples has been addressed by the SAB Temporary Working Group (TWG) on Sampling and Analysis.[19] The TWG recommended two methods of identification should be used, one essentially for screening, and the second for confirmation. Lateral flow immunoassays (LFA), qualitative ELISA and LC with fluorescence detection were recommended as screening assays, as widely used in the food and fishing industries,

---

[13] The military symbol TZ was derived after the name of its principal investigator, Dr Edward Shantz, who spent three decades working on toxins at the US Army Fort Detrick laboratory before joining the University of Wisconsin in 1972.

[14] The M1 Biodart (E1) was a 7.62 mm rifle cartridge flechette system filled with either Botulinum toxin A (XR), saxitoxin (TZ), or possibly a combination of the two. There were reportedly 4,450 filled and 5,315 unfilled M1s in the US arsenal just prior to their destruction in the early 1970s. (Information from wikipedia).

[15] WHO, Public health response to biological and chemical weapons, (World Health Organization, Geneva, 2004).

[16] USA, CD/500, (1984)

[17] R.J. Mathews, 'Saxitoxin and the CWC: Personal Recollections and Reflections', Annex 4 in Report of the Thirteenth Session of the Scientific Advisory Board, SAB-13/1 (1 April 2009).

[18] The issue of what constitutes saxitoxin shows again that the CAS registry numbers given in the Convention cannot be considered to have regulatory power. They are essentially identification aids. See Paragraph 4.4 in Report of the Eighth Session of the Scientific Advisory Board, SAB-8/1 (19 February 2006).

[19] SAB-16/1 Annex 2.

1H-NMR is also appropriate if obtainable. For confirmation the TWG recommended liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) or with high resolution mass spectrometry (LC-HRMS).

- - - o - - -