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Laboratory desalination experiments with some algal toxins

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ABSTRACT

Over the last several decades, countries throughout the world have experienced an escalating and worrisome trend in the incidence of harmful algal blooms (HABs). A concern is that highly potent algal toxins might be retained in the treated water, posing a threat to human health. Seawater contaminated with saxitoxins, domoic acid, okadaic acid, and brevetoxins was desalinated using small (<100 mL capacity) reverse osmosis and distillation equipment. Analyses of desalinated water samples indicated efficient removal of the four toxins to greater than 99%, except brevetoxins for which some carry-over was observed during distillation. Hypochlorite concentrations of 4 ppm or higher were sufficient to react with all of the saxitoxins, domoic acid and okadaic acid in the samples that contained initial toxin concentrations up to 1250 ng mL^{-1}. Brevetoxins appeared to be unaffected in experiments in which the toxins were exposed to up to 30 ppm hypochlorite in seawater at 35 °C for 60 min. These results and their implications in terms of desalination plant design and operation are discussed.

1. Introduction

An emerging threat to the desalination industry is the increasing occurrence of harmful algal blooms (HABs), commonly known as red tides. These microscopic algae can proliferate to form widespread high concentrations of cell mass. Although algal blooms take many forms and have diverse impacts [1], there are two general categories. Non-toxic but high biomass blooms, and toxic HABs that can have a low or high biomass. One potential effect of high biomass HABs on desalination plants is that the cells can restrict flow by clogging filters, but other impacts include fouling of surfaces due to particulate and dissolved organic materials that can also compromise the integrity of reverse osmosis (RO) membranes. Toxic HABs result from the potential of certain bloom-forming algal species to produce potent toxins which can cause morbidity and mortality in humans, marine mammals, fish, or other animals exposed to the toxins during food web transfer. The fate of HAB toxins during desalination treatment has only recently been recognized as a concern as the high potency of some of these compounds and their varied chemical structures and volatility suggest that some might persist in desalinated water, posing a threat to human health [2].

Reliable data that could provide an estimate of this threat has not been published due to the intermittent occurrence of algal blooms and a general absence of routine testing for algal toxins with sensitive analytical procedures. Also, desalination plants differ one from another in details of their design and operation so that broad conclusions based on industrial-scale observations may be unreliable. Alternatively, useful data can be obtained from relatively small-scale laboratory experiments in which the general conditions involved in desalination are simulated. Rather than rely on the occasional occurrence of natural blooms a variety of toxins with diverse molecular properties can be studied with a wide range of concentrations.

The toxins in this study represent four of the most potent and well-characterized groups of HAB toxins known from studies of shellfish poisoning (Fig. 1). The most potent of the HAB toxins are the saxitoxins which are responsible for paralytic shellfish poisoning (PSP). There are more than 30 identified congeners in nature [3] with different specific toxicities. Saxitoxin is the most toxic low molecular weight marine compound currently known [4] and is among the most potent natural poisons. They are heat stable with molecular weights in the range 250–500 Da, and thus should be excluded by desalination with reverse

Abbreviations: LC, Liquid chromatography; MS, Mass spectrometry; ELISA, Enzyme-linked immunosorbent assay; STX, Saxitoxin; PSP, Paralytic shellfish poisoning; ASP, Amnesic shellfish poisoning; DSP, Diarrhetic shellfish poisoning; NSP, Neurotoxic shellfish poisoning; RO, Reverse osmosis; MSF, Multistage flash evaporation.

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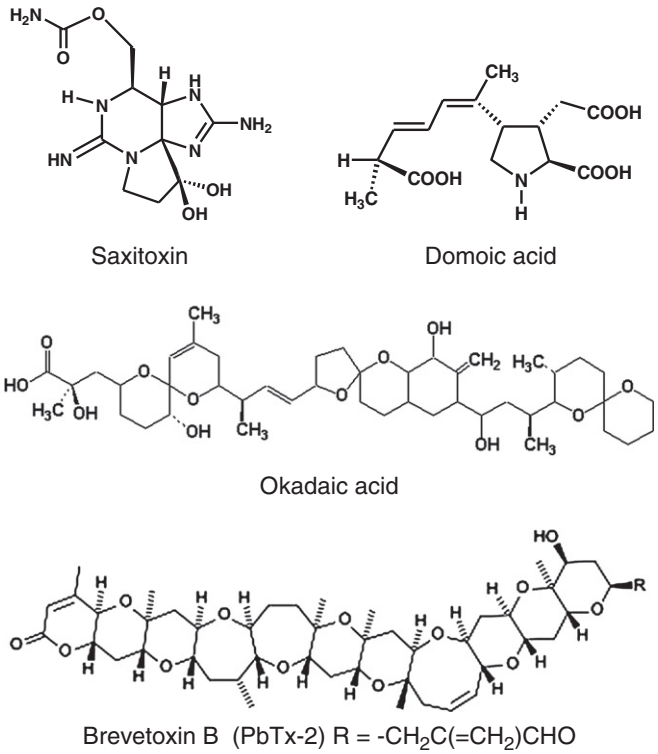

Fig. 1. Chemical structures of HAB toxins.

osmosis (RO) and distillation. Outbreaks of PSP result in more than 2000 illnesses worldwide each year, with a 5–10% mortality rate (Hallegraeff, 2003). Saxitoxins also have adverse effects on marine wildlife that can cause mortalities among fish, marine mammal and seabird populations [5].

Domoic acid is produced by some diatoms of the genus *Pseudo-nitzschia* [6]. Its specific toxicity is relatively low compared to saxitoxin. Cultured blue mussels (*Mytilus edilus*) from Prince Edward Island, Canada [7] contaminated with domoic acid poisoned 107 people and resulted in three fatalities. Domoic acid is soluble in water, non-volatile, and heat stable with a molecular weight of 312 Da [8], so it should be efficiently excluded by desalination processes.

Okadaic acid and the dinophysistoxins, DTX1 and DTX2, cause gastrointestinal distress, as part of a syndrome known as diarrhetic shellfish poisoning (DSP) [9]. In addition, okadaic acid has been reported to be a tumor promoter [10]. Okadaic acid and dinophysistoxins are produced by some species of the dinoflagellate genus *Prorocentrum* [11] and most commonly by some *Dinophysis* species. Although okadaic acid is a polyether, the molecule has a carboxyl group making it slightly soluble in water. With a molecular weight of 805 Da it is non-volatile and heat stable and should be removed by intact RO membranes and by distillation.

Brevetoxins are lipid soluble polyethers with no charged groups [12]. They have molecular weights around 900 Da and should be retained by properly functioning RO membranes. Brevetoxins have not been fatal to humans but cause a condition known as neurotoxic shellfish poisoning (NSP). The effects of brevetoxins on human health are well documented along the western coast of Florida in the U.S., where severe, nearly annual, red tides caused by the dinoflagellate *Karenia brevis* release large amounts of aerosolized brevetoxins into the air when the fragile cells are broken in breaking waves at the water's edge [13]. This aerosolization characteristic may result in carry-over in a multistage flash evaporation (MSF) desalination plant.

An important aspect of desalination is the pretreatment of intake water. Chemical treatments such as chlorination are generally used to kill microorganisms. [14]. However, little is known about the effects of chlorination on HAB toxins. Nicholson et al. (2003) [15] showed that hypochlorite can detoxify saxitoxins, but their study did not include other marine HAB toxins. Here we examine the fate of the four most prevalent marine HAB toxins during desalination and hypochlorite treatment using laboratory cultures, purified toxins, and small-scale laboratory apparatus. The implications of our results are then discussed in the context of desalination plant operation that could be implemented to mitigate effects of algal blooms.

2. Materials and methods

2.1. Toxins

The four toxins in this study were chosen because they are the most common HAB toxins and exhibit a wide range of solubility and molecular size. They were also available in adequate quantities for the laboratory experiments, together with their respective antibodies and conjugates for quantitative analyses by ELISA.

As a source of PSP toxins, a contaminated seawater sample was obtained from a 200 L culture of *Alexandrium tamarense*. Cells were harvested by filtration through Nitex 20 cloth, followed by centrifugation of the concentrate. Saxitoxin and neosaxitoxin were found in the supernatant fluid (120 mL) in approximately equal proportions. This solution contained 1.2 mg of the mixed PSP toxins and was subsequently spiked with domoic acid (2.0 mg) and okadaic acid (0.5 mg) and used for the RO and distillation experiments. It is hereafter referred to as the 'multiple toxin seawater' sample. Domoic acid was purchased from BioVectra Inc., Prince Edward Island, Canada and okadaic acid was purchased from LC Laboratories, Boston MS, USA. The source of brevetoxins was the supernatant fluid (600 mL) obtained by growing a laboratory culture of *Karenia brevis.* After sonication and centrifugation the solution contained 900 ng mL^{-1} of brevetoxins, the predominant toxin being PbTx-2.

2.2. Desalination apparatus

A small reverse osmosis desalination unit (Katadyne. RO6) was purchased from West Marine Inc. and is sold as emergency equipment for use in life-boats. The polyamide SW 30 membrane is custom made for Katadyne by Dow FilmTec. Pressure was provided with a manually operated pump as part of the unit. The volume on the seawater side was 75 mL and when pumped normally from seawater (salinity; 25 g L^{-1}) 150 mL was produced in 10 min with a salinity of 0.7 g L^{-1} (97.2% desalination). A glass micro-distillation apparatus was purchased from Laboy, Beijing, China. The distillation flask had a maximum capacity of 50 mL and 10 mL for each of the three collection vessels. From 30 mL of the spiked seawater three 4 mL fractions were collected.

2.3. Analytical methods

For saxitoxin, domoic acid, and okadaic acid analyses, an indirect competitive ELISA procedure was used. Polyclonal antibodies and toxin-BSA (bovine serum albumin) conjugates were obtained from Jellett Rapid Testing Ltd and calibration standards were purchased from the National Research Council of Canada. Wells were coated with toxin conjugates. The sample or standard was then added to the wells together with rabbit polyclonal anti-toxin and incubated for 1 h at 37 °C. Rabbit antibodies were detected using goat anti-rabbit antibody with conjugated horseradish peroxidase (GAR-HRP). Incubation was for 1 h at 37 °C followed by washing five times with PBS. The enzyme substrate was *o*-phenylenediamine (OPD) and hydrogen peroxide with incubation at room temperature for 5 min. Optical densities at 490 nm were measured with a microtiter plate reader and plotted with the Soft Max Pro program. The ELISA method according to Naar et al. [16] was used for analysis of brevetoxins.

2.4. Experiments with hypochlorite solutions

Preliminary experiments were conducted to investigate the effect of hypochlorite on the toxins using ELISA to analyze solutions after incubation with different concentrations of hypochlorite. A fresh commercial solution of chlorine bleach had an indicated concentration of 6% hypochlorite from which a working solution of 60 mg L^{-1} was prepared by dilution 1:1000 in seawater (salinity 45 g L^{-1}). Dilution series were prepared in which hypochlorite concentrations decreased by a factor of two at each step. Three separate toxin solutions were prepared in seawater, each containing 2500 ng mL^{-1} of STX.2HCl mL^{-1}, domoic acid, or okadaic acid. An equal volume of each toxin solution was added to the different hypochlorite solutions in the series so that 1250 ng mL^{-1} of toxin was exposed to hypochlorite at different concentrations and incubated for 10 min at 37 °C. Preliminary experiments had shown that the tests functioned normally with up to 30 ppm hypochlorite having no detectable effect on the antibodies or on the toxin bound to the microtiter plates. Experiments with brevetoxins were conducted separately and toxin concentrations are given with the ELISA results.

In addition to ELISA, LC-MS/MS analyses were conducted to confirm degradation of toxins in hypochlorite solutions and to look for degradation products. An Agilent model 1100 HPLC coupled to an AB-SCIEX API-4000 triple quadrupole mass spectrometer (Waltham MA, USA) equipped with a TurboSpray® interface was used. The HPLC included an in-line degasser, binary pump, refrigerated autosampler and temperature-controlled column oven. Saxitoxins were analyzed with a TSK-Gel Amide-80 column (250 mm × 2 mm i.d., 5 μm) (Tosoh Bioscience LLC, Montgomeryville, PA, USA) at 40 °C [17]. An isocratic mobile phase was used with 35% solvent A (water with 2.0 mM ammonium formate, and 3.6 mM formic acid) and 65% solvent B (95% acetonitrile) at a flow rate of 0.2 mL min^{-1}. Domoic and okadaic acids were analyzed using a Synergi Polar-RP column (50 mm × 2 mm i.d., 2 μm) (Phenomenex, Torrance, CA, USA) maintained at 40 °C. The mobile phase was comprised of solvent A (water) and solvent B (95% acetonitrile) both with 2.0 mM ammonium formate and 50 mM formic acid at a flow rate of 0.2 mL min^{-1}. The run started with an isocratic period of 5 min at 100% A, followed by a gradient to 100% B over 15 min, a hold at 100% B for 5 min. After returning to initial conditions a re-equilibration time of 13 min was used. Samples were prepared by diluting 50 μL of standard toxins in 450 μL fresh seawater with either 0 or 10 ppm hypochlorite. The final concentrations of saxitoxin, domoic acid and okadaic acid were 2.0, 10.0, and 1.4 μg mL^{-1}, respectively. Another experiment with a higher concentration of STX (25 μg mL^{-1}) was also conducted in an effort to find degradation products. Seawater with 10 ppm hypochlorite solution diluted to the same extent was used as a blank control. All of these samples were heated in an oven at 37 °C for 10 min before analysis.

3. Results

3.1. Desalination experiments with PSP, ASP, and DSP toxins

The seawater sample (120 mL) containing high concentrations of PSP, ASP, and DSP toxins was prepared as described above in Section 2.1. In order to simulate seawater that would occur in warm waters it was necessary to add sea salt (Instant Ocean ™) to increase the salt concentration to 37–45 g L^{-1}. Initial toxin concentrations were: saxitoxins at 10,300 ng mL^{-1}, domoic acid at 17,000 ng mL^{-1}, and okadaic acid at 400 ng mL^{-1}.

3.1.1. Desalination by reverse osmosis (RO)

Preliminary experiments with the RO unit showed the volume of seawater required to fill the apparatus was 75 mL. A greater volume was necessary to allow recycling through the seawater intake and outlet tubes (Fig S2) from a reservoir and this was 120 mL. This minimum volume allowed high toxin concentrations to be tested. In a second experiment a sample was diluted to avoid the need to recycle and provide more typical natural toxin concentrations. The toxin concentrations were reduced to 512 ng mL^{-1} for saxitoxins, 650 ng mL^{-1} for domoic acid, and 20 ng mL^{-1} for okadaic acid. The volume of collected desalinated water was limited to 5 mL in order to avoid significantly increasing concentrations of salt and toxins in the seawater. Although the salt concentration of the permeate (i.e., treated) sample in the first experiment was 2.5 g L^{-1}, which represented a desalination efficiency of only 93%, toxin elimination was greater than 99% (Table 1).

Toxin concentrations in the first RO desalinated sample were 61 ng mL^{-1} for saxitoxins and 165 ng mL^{-1} for domoic acid. Okadaic acid was below the limit of detection (2 ng mL^{-1}) for the assay. This means that at least 99.7% of the okadaic acid was excluded by the RO membrane. The 0.60% carry-over of saxitoxins and 0.96% for domoic acid indicated that 99.4% of the saxitoxins and 99.0% of the domoic acid were excluded by the RO membrane (Table 1).

For the repeat RO experiment the seawater salt concentration was similar to that in the first experiment, but desalination was much more efficient without recycling (Table 1. PSP2 and ASP2). Also, the initial toxin concentrations were considerably lower as stated earlier. After desalination the salt concentration was 0.28 g L^{-1} compared to 2.5 g L^{-1} in the first experiment. Elimination of saxitoxins was at least 99.6% and better than 99.2% for domoic acid.

3.1.2. Desalination by distillation

Three consecutive 5 mL samples of distillate were collected from an initial volume of 30 mL of the multiple toxin seawater sample containing PSP, ASP, and DSP toxins in the boiling flask. The salinity of the first fraction was 0.5 g L^{-1} and for subsequent fractions 0.2 g L^{-1}. None of the toxins were detected by ELISA indicating negligible carry-over by distillation (Table 2).

3.2. NSP toxins (brevetoxins)

For the first experiments with RO and distillation the initial sample in seawater (600 mL) contained 900 ng mL^{-1} PbTx-2 and PbTx-3. The toxic seawater sample was prepared from a flask culture of *Karenia brevis* with 25×10^6 cells L^{-1} and 40 pg $cell^{-1}$. In natural blooms cell densities often occur within the range 0.1 to 5×10^6 cells L^{-1} and occasionally up to 100×10^6 cells L^{-1}.

3.2.1. Desalination by reverse osmosis

The salinity of the sample was 32.5 g L^{-1}. After desalination, salinity of the permeate was 0.25 g L^{-1} which represents 99.2% elimination of salt. No brevetoxins were detected in the permeate. With a sensitivity of 1 ng/mL toxin elimination was, therefore, at least 99.9% (Table 1).

3.2.2. Desalination by distillation

With an initial volume of 30 mL in the distillation flask, three consecutive samples each of 4 mL were collected and labeled Dist1, Dist2, and Dist3. The maximum temperature in the flask was 104 °C. The final brine volume after distillation was 18 mL from the original

Table 1
Summary of reverse osmosis experiments (% exclusion in parentheses).

Seawater			Permeate	
	Toxin ng mL^{-1}	Salt g L^{-1}	Toxin ng mL^{-1}	Salt g L^{-1}
PSP1	10,340	37	61(99.4)	2.5 (92.2)
PSP2	512	32	<2 (99.6)	0.28 (99.1)
ASP1	17,150	37	165 (99.0)	2.5 (93.2)
ASP2	650	32	<5 (99.2)	0.28 (99.1)
DSP	400	37	<2 (99.7)	2.5 (93.2)
NSP	900	32	<0.5 (99.9)	0.25 (99.2)

Table 2
Summary of distillation experiments (% exclusion in parentheses; ND not determined).

Seawater			Distillate	
	Toxin ng mL^{-1}	Salt g L^{-1}	Toxin ng mL^{-1}	Salt g L^{-1}
PSP	10,340	37	<2 (99.9)	0.2 (99.4)
ASP	17,150	37	<5(99.9)	0.2 (99.4)
DSP	400	37	<2 (99.5	0.2 (99.4)
NSP	900	32	<15 (98.3)	0.008 (100)
NSP (A)	462	ND	8 (98.2)	ND
NSP (B)	954	ND	25 (97.3)	ND

30 mL. Dist1 contained 42 ng, Dist2 102 ng, and Dist3 32 ng making the total amount of toxin that distilled over 176 ng in a combined volume of 12 mL. This is shown in Table 2 as the average of the three fractions (15 ng mL^{-1}). The total carry-over of toxin was 176 ng out of the initial amount of 27,000 ng in the distillation flask and represents 0.65%. Two repeat experiments were performed (A and B in Table 2) for which 1.7% and 2.6%, respectively, of the initial brevetoxins in the distillation flask were found in the (omitted freshwater) distillates.

3.3. Effects of hypochlorite

Data from experiments in which the four HAB toxins were exposed to hypochlorite concentrations up to 30 ppm in seawater at 35 °C for between 10 and 60 min are summarized in Fig. 2. Measurements with ELISA assays clearly showed that hypochlorite concentrations of 4 ppm or higher were sufficient to degrade all of the saxitoxin, domoic acid, and okadaic acid in the samples that contained initial toxin concentrations up to 1250 ng mL^{-1} (Fig. 2). Domoic acid was the most sensitive of the four toxins requiring only 1 ppm hypochlorite. Brevetoxins appeared to be unaffected in experiments in which the toxins at two different concentrations were exposed to up to 30 ppm hypochlorite in seawater at 35 °C for 60 min. The low toxin concentration contained PbTx-3 at 3 ng mL^{-1}. The higher concentration (approx. 300 ng mL^{-1}) consisted of a mixture of brevetoxins in a sonicated and filtered culture of *Karenia brevis*.

LC-MS/MS analyses were also performed, using the very sensitive selected reaction monitoring (SRM) mode, on seawater samples spiked with saxitoxin, domoic acid and okadaic acid before and after a 10 min reaction with 10 ppm hypochlorite. These toxins were no longer detectable after the reaction (<1% of original signal without hypochlorite). Full Q1 scans, neutral loss scans and precursor scans were used to try to detect any degradation products. No products could be detected with saxitoxin, even when higher concentrations were used. Reactions in distilled water were also monitored in case the seawater was causing suppression of signals, but no products were detected in that case either. For domoic acid, one significant product comparable in concentration to the original toxin was detected. It had a molecular weight of 217 and gave an MS/MS product ion spectrum more complex than that of domoic acid. Several new compounds were found in the okadaic acid reaction: these were two isomeric unknown compounds with molecular weight 947 and another four compounds with molecular weight 993. The isotope cluster pattern for the $[M+H]^+$ ions indicated that these all contained 4 chlorine atoms. Product ion spectra of these compounds were quite different than that of okadaic acid.

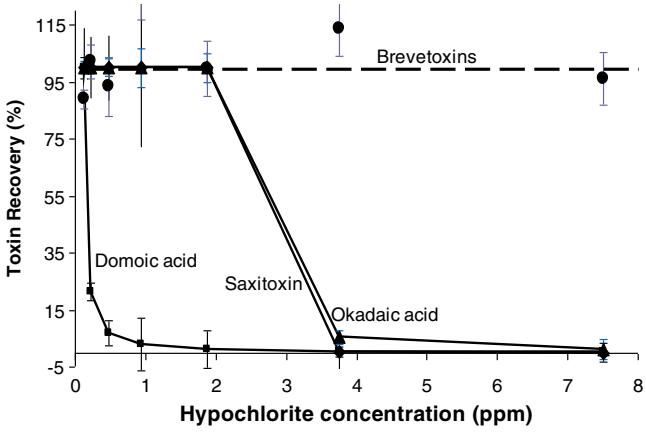

Fig. 2. Effect of hypochlorite on HAB toxins as determined by assays. The three common HAB toxins, saxitoxin (STX), domoic acid (DA), and okadaic acid (OA) each at 1250 ng mL^{-1}, were exposed to a range of hypochlorite concentrations up to 30 ppm in seawater (45 g L^{-1}) at 35 °C for 10 min. Exposure time was limited to 10 min to avoid interference of hypochlorite with antibodies and bound toxins in ELISA experiments. With hypochlorite concentrations of up to 4 ppm the toxins were no longer recognized by their respective antibodies, indicating a chemical change. Experiments with brevetoxin concentrations at 3 ng mL^{-1} PbTx-3 and a second experiment with a mixture at 300 ng mL^{-1} were exposed to hypochlorite concentrations up to 30 ppm for 60 min at 37 °C. Brevetoxin concentrations measured by ELISA remained around 100% (dashed line), indicating no effect.

4. Discussion

In regions where most freshwater is produced by desalination water quality has a high priority, especially for drinking water. Other considerations are the cost of production and reliability of supply. A potential problem that is not so well understood, however, is that some phytoplankton blooms produce potent toxins that could contaminate drinking water. The results of our experiments indicate that the risk appears to be small. Even with unusually high concentrations of four of the most dangerous shellfish toxins, their elimination by both reverse osmosis and by simple distillation was greater than 99% in the laboratory experiments. Although the data are limited to four HAB toxins (Fig. 1), they are the most likely to be present in blooms.

The results of the RO experiments (Table 1) indicate that all four toxins were effectively removed from the drinking water to greater than 99%, even with toxin concentrations that were unusually high. Saxitoxins in the seawater sample were present at 10,340 ng mL^{-1}. An unusually dense bloom of 10^7 cells L^{-1} and toxin concentration of 40 pg $cell^{-1}$ is equivalent to 400 ng mL^{-1}. Therefore, the saxitoxin concentration was twenty five times that of an unusually high bloom concentration. The concentration of the ASP toxin, domoic acid, was even higher at 17,150 ng mL^{-1}, which would be about forty times that of a dense bloom concentration. For the repeat RO experiments with 512 ng mL^{-1} of saxitoxins and 650 ng mL^{-1} of domoic acid the toxin concentrations in the treated water were below the limit of detection. This was also the case for the permeate concentrations of DSP and NSP toxins.

For the distillation experiments (Table 2) it was not necessary to repeat the saxitoxin, domoic acid, and okadaic acid treatments, because even with the high seawater toxin concentrations carry-over into the distillates were all below the limits of detection (Table 2). However, this was not so for the NSP distillation experiments where the average toxin removal was 98% indicating that 2% of the initial brevetoxins were found in the treated water. The highest brevetoxin concentration was 954 ng mL^{-1}, which could occur in a bloom of 10^8 cells L^{-1} with 10 pg $cell^{-1}$. Because brevetoxins are known to be carried inland in Florida as aerosols from red tides [18] it was predictable that brevetoxins might contaminate drinking water prepared by a desalination process based on distillation.

Risk assessments for the common groups of marine toxins to the shellfish industry were summarized recently [19]. Using these data the potential threat for desalinated water can be estimated.

The European Union Food Safety Authority has established three levels of risk characterization from epidemiological data [20].

1. Lowest observed adverse effect level (LOAEL).
2. No observed adverse effect level (NOAEL).

3. Acute reference dose (ARfD). The estimated amount that can be ingested in 24 h without health risk. This is obtained from 1 and 2 by dividing by safety factors. It is the most appropriate risk level to apply to drinking water.

For the saxitoxin group, the EU ARfD = 0.5 μg STX equiv kg^{-1} body weight. For domoic acid, the ARfD = 100 μg kg^{-1} body weight, and for the okadaic acid group, the ARfD = 0.33 μg kg^{-1} body weight. For the brevetoxin group, an ARfD has not yet been established. However the regulatory limit for shellfish is the same as for saxitoxins (80 μg STX equiv 100 g^{-1} shellfish tissue in the mouse bioassay) so we could assume an ARfD of 0.5 μg kg^{-1} body weight. An algal bloom can often be seen as a discoloration (red tide) when cell concentrations exceed 10^5 cells L^{-1}. A dense bloom may have 10^7 cells L^{-1}, together with a high toxin concentration of 40 pg $cell^{-1}$. Table 3 shows the volumes of drinking water that would have to be consumed in 24 h to exceed the EU ARfD for each of the toxins. Considering the large volumes of water that would have to be consumed and the conservative ARfD values, the probability that anyone would suffer harmful effects from drinking water produced by desalination of HAB contaminated seawater is extremely low. This is assuming desalination operations are consistently efficient. Given the possibility for failed membranes or other problems in the treatment process and recognizing the high potency of HAB toxins, it may well be prudent for desalination plants to perform regular monitoring of phytoplankton and to initiate chemical treatment (chlorination) of intake water during a bloom of known toxic species.

In many desalination plants, chlorine compounds such as sodium or calcium hypochlorite, chlorine gas or chlorine dioxide are added at approximately 1 ppm (with occasional pulses to higher concentrations) (K.P. Abraham, Pers. Comm.). Chlorination is mainly used to partially sterilize input water by killing bacteria and to dissolve gelatinous polymers associated with bacteria. The effect on algal cells and toxins has not, until recently, been a major consideration except to minimize restricted flow [21]. Hypochlorite is known to detoxify saxitoxins [15] but degradation products were not identified. It is known that saxitoxins are readily oxidized to fluorescent derivatives when exposed to oxidizing agents [22] but none of these were detected in our experiments. It has been reported that domoic acid is susceptible to oxidative conditions, particularly through UV-promoted reactions [23], but reactions with hypochlorite have not been reported to our knowledge.

Our ELISA tests of reactions of the four toxins with various concentrations of hypochlorite indicated that only brevetoxins were unaffected by up to 30 ppm hypochlorite (Fig. 2). LC-MS analyses confirmed that saxitoxin, domoic acid and okadaic acid were completely destroyed by exposure to 10 ppm hypochlorite at 37 °C for 10 min.

No decomposition products were detected by LC-MS for the saxitoxin-hypochlorite reaction, so it is probable that the saxitoxin was extensively degraded to low molecular weight substances, which are not likely to be toxic. A significant, single unknown degradation product of domoic acid with a molecular weight of 217 was detected by LC-MS indicating extensive structural changes. As stated above, given the possibility for failed membranes or other unanticipated problems in the treatment process and recognizing the high potency of HAB toxins, it may well be prudent for desalination plants located in HAB-prone regions to perform regular monitoring of phytoplankton and to initiate chemical treatment (chlorination) of intake water during a bloom of known toxic species.

Because HAB events are unpredictable and HAB toxins are not generally monitored at desalination plants, investigations of possible contamination of drinking water from large blooms has not been conducted by the industry. A single pilot-scale study documented the removal of a domoic acid analog using RO [24]. Laboratory-scale experiments have provided an opportunity to investigate this problem using small volumes of seawater spiked with high concentrations of four of the major HAB toxins. Except for the brevetoxins toxin removal was in excess of 99% indicating that, even with an intense bloom, there is very little threat to human health if plant operations are efficient. Given concerns about operational mistakes or breakdowns, however, a prudent step would be to establish an “adaptive” HAB toxin testing capability, guided by regular phytoplankton monitoring that would detect potentially toxic species, as well as those that are non-toxic, but that could clog filters or cause other problems due to high biomass effects. This would provide plant managers with data to make decisions about chemical treatment, or ultimately, when to suspend production in the interests of safety.

Table 3
Risk assessment. The data show volumes of drinking water if consumed by a 60 kg person in a 24 h period would equal the European Union ARfD amounts for different HAB toxins. The assessment is based on a hypothetical bloom of toxic algae consisting of 10^7 cells L^{-1} and 40 pg toxin $cell^{-1}$. Total toxin release from cells would give a concentration in seawater of 400 μg L^{-1}. With an assumed desalination efficiency of 99% for toxin elimination the concentration in drinking water would be 4 μg L^{-1}.

	Toxin (μg)	Volume (L)
PSP	42	10.5
ASP	6000	1200
DSP	20	5.0
NSP	42	10.5

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.desal.2012.02.014.

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