Direct Competitive Enzyme-Linked Immunosorbent Assay for Saxitoxin and Neosaxitoxin

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Using specific ploYclonal antibodies against saxitoxin (STX) or neosaxitoxin (neo-STX) in combination with either STX—horseradish peroxidase (HRP) or neo-STX—HRP, the efficacy of four different direct competitive enzyme-linked immunoassorbent assay (dc-ELISA) formats for the analysis of STX and neo-STX was evaluated. Concentrations causing 50% inhibition (ID_{50}) of binding of toxin—HRP conjugate to the antibodies by free toxins in various ELISAs were found in the range of 0.1—9.0 ng/mL. A dc-ELISA, using either anti-STX/STX—HRP or anti-neo-STX/neo-STX—HRP pairs (ID_{50} values of 0.28 and 0.18 ng/mL for STX and neo-STX, respectively), was found to be most effective for the analysis of STX and neo-STX in naturally contaminated shellfish samples. The analytical recoveries of STX added to viscera extracts of butter clams, dungeness crab, tanner crab, and blue mussels in the range of 0.5—10 ng/mL^{-1} g^{-1} were found to be 88.1, 92.7, 92.2, and 93.5% with coefficients of variation of 3.9, 2.7, 9.7, and 2.8%, respectively. The detection limit for STX and neo-STX in these shellfish was around 0.2 ng/g of tissue. Gonyautoxins 1—4, but not the C group of PSP toxins, were also detectable in these two systems. Analysis of 154 naturally contaminated shellfish samples showed good correlation between the ELISA (STX plus neo-STX levels) and mouse assay data. The data reported here suggest that simultaneous analysis of both STX and neo-STX by ELISA is necessary for accurate determination of overall PSP toxin levels.

Keywords: *ELISA; saxitoxin; neosaxitoxin; PSP; immunoassay*

INTRODUCTION

Paralytical shellfish poisoning (PSP) toxin is one of the most potent naturally occurring food poisonings. The poison constitutes a group of toxins produced predominantly by the dinoflagellate *Alexandrium (= Protogonyaulax) catenella* and *Alexandrium tamarense* and is primarily encountered in toxic mussels, clams, and other marine animals. Saxitoxin (STX) is one of the major and most potent members in this group of toxins, and at least 20 analogues of STX have been reported (Anderson, 1994; Hall et al., 1990; Schantz, 1979). In addition to dinoflagellates, the freshwater cyanobacterium (blue-green alga) *Aphanizomenon flos-aquae* is also known to produce STX and neosaxitoxin (neo-STX) (Mahmood and Carmichael, 1986). The toxins involved in PSP tend to have little adverse effect on the shellfish using the dinoflagellates as a food source. However, human ingestion of toxin-contaminated shellfish may result in paralytic shellfish poisoning, which can be fatal (Anderson, 1994; Boyer et al., 1979; Hall et al., 1990).

Because of the potential health hazard, a quick, sensitive, and specific method is needed to determine the presence of toxins in shellfish. Several methods, including mouse bioassays (Hollingworth and Wekell, 1990), receptor binding assay (Davio and Fontelo, 1984; Smith and Kitts, 1994; Vieytes et al., 1993), tissue culture assays (Gallacher and Birkbeck, 1992; Jellett et al., 1992, 1995; Manger et al., 1993), and chemical methods, have been used for the analysis of PSP toxins (Hollingworth and Wekell, 1990). Details of these methods have recently been reviewed (Lucas, 1992; Hungerford and Wekell, 1992). However, the chemical methods, such as HPLC (Lawrence and Menard, 1991; Lawrence et al., 1991; Lucas, 1992; Oshima, 1995; Sullivan and Iwaoka, 1983; Sullivan et al., 1988), capilliary electrophoresis (CE) (Thibault et al., 1991; Pleasance et al., 1992a,b), and HPLC/MS and CE/MS (Mirocha et al., 1992; Pleasance et al., 1992a,b), involve considerable effort for sample treatment and derivatization and require expensive instrumentation. Although most biological methods are very simple to perform, they lack specificity. With the availability of antibodies against STX and neo-STX, several immunoassay protocols (Hokama and Smith, 1990), e.g. hemagglutination (Johnson et al., 1964), radioimmunoassay (Carlson et al., 1984; Chu and Fan, 1985) and enzyme-linked immunoassays (ELISA) (Cembella et al., 1989; Chu and Fan, 1985; Chu et al., 1992; Renz and Terplan, 1988; Usleber et al., 1991), have been developed. Monoclonal antibodies against STX (Hack et al., 1990; Hout et al., 1989) have been produced. Commercial ELISA kits are also available (for example, Inst. Armand-Frappier, Laval, PQ, Canada, and R-Biopharm, GmbH, Darmstadt, Germany). However, antibodies used in these ELISAs are very specific. For example, antibodies against STX have little cross-reaction with other STX-related PSP toxins. Their cross-reactivity with neo-STX, also frequently involved in PSP, was only 2—10% that of STX (Cembella et al., 1989; Chu and Fan, 1985; Renz and Terplan, 1988). Attempts to overcome such deficiency were made in our laboratory by generating polyclonal antibodies against neo-STX with increased cross-reaction with STX (Chu et al., 1992). Depending upon the ELISA formats used, the cross-reactivity of these antibodies with STX varied from 10 to 50% of that of neo-STX (Chu et al., 1992). However, the use of these antibodies for the analysis of PSP toxins in naturally occurring outbreak samples still could

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result in an underestimation of total toxin concentrations. With the availability of two types of antibodies with a varied range of cross-reactivity with STX and neo-STX, the efficacy of four direct competitive ELISA (dc-ELISA) formats, involving the use of two different antibodies and two enzyme−toxin conjugates, was evaluated in the present study. We have found that two of the four dc-ELISA protocols tested are very effective for the analysis of STX and neo-STX. Details of various ELISA protocols, the sensitivity of different methods, the analysis of solutions containing three major groups of PSP toxin, and the analytical recovery of STX added to four different shellfish by a dc-ELISA are presented. To evaluate the practical application of these ELISA protocols for the analysis of PSP, we also analyzed a total of 154 naturally contaminated shellfish samples, including viscera from 58 Dungeness crabs, 47 Tanner crabs, 36 blue mussels, and 13 oysters, which had been previously analyzed for PSP toxin by mouse assay.

MATERIALS AND METHODS

Materials. Purified STX was kindly provided by Drs. E. J. Schantz (Food Research Institute, University of Wisconsin−Madison) and R. W. Wannemacher, Jr. (U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrich, MD). Neo-STX was kindly provided by Dr. Sherwood Hall of the FDA (Washington, DC) and was prepared according to the method previously described (Hall et al., 1990). Bovine serum albumin (BSA, RIA grade) and horseradish peroxidase (HRP) (ELISA grade, catalog no. 605 220) were obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). Tween 20 and *o*-phenylenediamine (OPD) were obtained from Sigma Chemical Co. (St. Louis, MO). ELISA microwell plates were purchased from Nunc (high binding capacity, Nunc Co. no. 4-69914, Roskilde, Denmark). Antibodies against STX and neo-STX were prepared in our laborotory as previously described (Chu and Fan, 1985; Chu et al., 1992). All chemicals and organic solvents were of reagent grade or better.

Preparation of STX−HRP Conjugate. STX−HRP and neo-STX−HRP conjugates were prepared according to the reductive alkylation method after oxidation of HRP with $NaIO_4$ (Wilson and Nakane, 1978). In a typical experiment, 4 mg of HRP in 1 mL of distilled water was reacted with 0.2 mL of $NaIO_4$ solution (0.1 M) at room temperature for 20 min. The reaction mixture was then dialyzed against 2 L of sodium acetate buffer (pH 4.4, 1.0 mM) at 4 °C overnight. The solution was then separated into two portions, and 40 μg of either STX or neo-STX was then added to each portion with the pH of the solutions adjusted to 7.5 with 0.2 M sodium carbonate buffer. The reaction proceeded at 20 °C for 60 min, and then 60 μL of $NABH_4$ (4 mg/mL) was added and incubated at 4 °C for additional 30 min. The solution was then dialyzed against 0.01 M sodium phosphate buffer (PB) consisting 0.15 M NaCl (PBS, pH 6.0; changes, 2 L each) at 4 °C for 2 days. The toxin−HRP was then stored in glycerol (1:1 ratio) at −20 °C.

dc-ELISA. The protocol for the direct ELISA was essentially the same as we previously described for aflatoxin B_1 (Chu et al., 1987). Four different formats, including coating antibodies against STX and then using either STX−HRP or neo-STX−HRP as a marker and coating neo-STX antibodies and then using either of these conjugates as the marker, were tested. For coating of the antibody to the solid phase, 100 μL of the diluted antibody solution (57 μg of anti-STX or anti-neo-STX IgG/mL of 0.01 mol/L PBS, pH 7.5) was added to each well of a Nunc microwell plate and kept in a cold room overnight. In general, after the coated plate was incubated at 4 °C overnight, the plate was washed with PBS−Tween (0.35 mL/well; 0.05% Tween 20 in 0.01 M PBS, pH 7.5) in an automatic ELISA washer (Dynatech Model B miniwasher) followed by incubation with BSA−PBS (0.17 mL/well; 0.1% BSA in 0.01 M PBS, pH 7.5) at 37 °C for 30 min. The plate was washed again with PBS−Tween (0.35 mL/well) four times followed by addition of 0.05 mL of standard STX or neo-STX at different concentrations, blank buffer, or sample solution together with 0.05 mL of STX−HRP (0.25 μg/mL) or neo-STX−HRP conjugate (0.5 μg/mL) to each well. After incubation at 37 °C for 60 min, the plate was washed, and 0.1 mL of freshly prepared OPD substrate solution [10 mg of OPD plus 13 μL of 30% hydrogen peroxide in 25 mL of 0.05 M citrate−phosphate buffer (4.8 g of citric acid and 7.1 g of Na_2HPO_4 in 500 mL of distilled water with pH adjusted to 5.0)] was added. Ten minutes after incubation at room temperature in the dark, the reaction was terminated by adding 0.1 mL of 1 N HCl. Absorbance at 490 nm was determined in an automatic ELISA reader (THERMO/max microplate reader, Molecular Devices Co., Menlo Park, CA). Triplicate analyses were made for each sample.

Standard STX or neo-STX solutions were prepared as stock solution A (10 μg/mL of 0.1 N HCl) and stock solution B (1 μg/mL of 0.01 N HCl) and kept in the freezer. A series of standard solutions was prepared by diluting stock solution B to appropriate concentrations with PBS and used within 1 week. Because of the instability of the toxin at neutral and alkaline pH, the diluted solution stock solution B should be never kept for more than 1 month in the refrigerator.

Analytical Recovery Experiments. The analytical recovery experiments were conducted for the dc-ELISA of STX in butter clams, Dungeness crab, tanner crabs, and blue mussels. The sample extracts were supplied by Ms. Chris Allison and Dr. Dick Barrett of the Environmental Health Laboratory of the Department of Environmental Conservation of the State of Alaska. The sample extracts were prepared according to the standard AOAC method (Hollingworth and Wekell, 1990) and were shown to be negative for the PSP toxins in the mouse assay. They were also negative for STX and neo-STX as analyzed by ELISA in our laboratory. Appropriate amounts of STX standard were added to the shellfish sample extract to give the following final concentrations: 0.1, 0.5, 1, 2, 5, and 10 ng of STX/mL.

ELISA Analysis of Different Mixtures of PSP Toxins. To test the cross-reactivity of the antibodies with various PSP toxins, three sets of standard mixtures containing various types of PSP toxins that were kindly supplied by Dr. Gregrey Doucette of the National Marine Fisheries Service, Charleston, SC, were subjected to the dc-ELISA for both STX and neo-STX using the protocols as described above.

dc-ELISA for STX and Neo-STX in Naturally Occurring Samples. To test the efficacy of practical application of the dc-ELISA in the naturally contaminated samples, 154 sample extracts from the viscera of 4 different species of shellfish that had been shown to be positive in an indirect competitive ELISA and mouse assays were subjected to the ELISAs. Two types of ELISA, using antibodies either specifically against STX together with STX−HRP or anti-neo-STX together with neo-STX−HRP, were run for each sample. Thus, each assay determined the level of a specific toxin, i.e. either STX or neo-STX. Since the antibodies also cross-react with gonyautoxins (see data in Table 3), the levels of STX and neo-STX obtained from these two ELISAs represent almost all of the PSP toxins with the exception of C groups of PSP toxins because they reacted weakly with both types of antibodies. The toxin extracts were provided by Ms. Chris Allison as described above. Samples were collected from various locations in Alaska and were prepared and analyzed for PSP toxins by mouse assay according to the standard AOAC protocols in the Alaska Laboratory in 1994.

RESULTS AND DISCUSSION

Comparison of Four Different dc-ELISA Formats for STX and Neo-STX. The efficacy of four dc-ELISA formats for the analysis of STX and neo-STX, involving two types of antibodies and two different toxin−HRP conjugates with various combinations, was tested. Parts A and B of Figure 1 show the effect of free STX and neo-STX on the binding of STX−HRP or neo-STX−HRP to the solid-phase *anti-STX* or *anti-neo-*

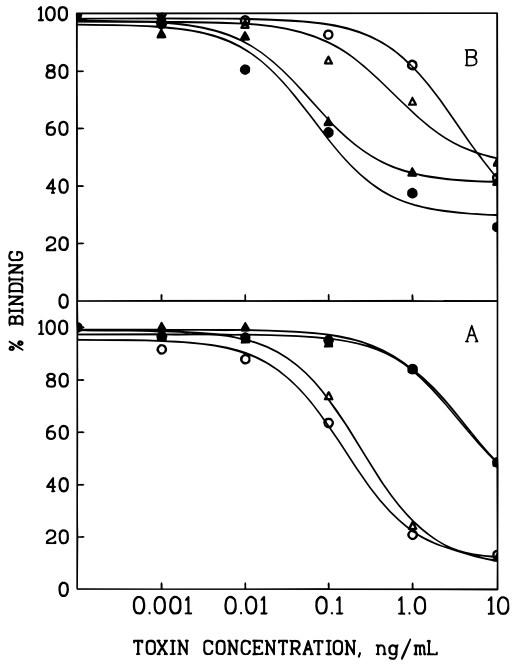

Figure 1. dc-ELISA and STX and neo-STX using antiserum specifically against STX (A) or neo-STX (B). One hundred microliters of the diluted rabbit anti-STX (A) or anti-neoSTX (B) antiserum (57 μg of IgG/mL) was coated to each well of the ELISA. Fifty microliters of either STX−HRP (2.5 μg/mL) or neo-STX−HRP (5.0 μg/mL) together with 50 μL of various concentrations STX or neo-STX was used in the test. The symbols represent the data obtained from the following combinations: STX with STX−HRP (△); neo-STX with STX−HRP (▲); STX with neo-STX−HRP (○); neo-STX with neo-HRP (●); open and solid symbols for systems used STX and neo-STX, respectively.

Table 1. Concentrations Causing 50% Inhibition (ID_{50} Values, Nanograms per Milliliter) of the Binding of Toxin−HRP Markers with the Solid-Phase Antibodies under Four Different ELISA Formats

	STX−HRP		neo-STX−HRP	
antibody used	STX	neo-STX	STX	neo-STX
anti-STX	0.28	8.89	0.18	9.04
anti-neo-STX	9.09	0.34	1.10	0.18

STX. The concentrations causing 50% inhibition (ID_{50} values) of the binding of *STX−HRP* or *neo-STX−HRP* to the *anti-STX* or *anti-neo-STX* antibodies by STX and neo-STX are given in Table 1. Data from these experiments clearly show that highest sensitivity of ELISA for STX or neo-STX was achieved when specific pairs of antibody and toxin marker are used. Figure 1B also shows that high sensitivity of ELISA for neo-STX could be achieved in the *STX−HRP* (marker)-*anti-neo-STX* antibody system. A series of experiments, using various combinations of anti-STX or anti-neo-STX antibodies together with STX−HRP or neo-STX−HRP, was also conducted. Consistent data were obtained when *STX−HRP* was used as the marker in two separate ELISAs in combination with either anti-STX or anti-neo-STX antibodies coated to the ELISA plate (data omitted).

Standard Curves for dc-ELISA of STX and Neo-STX. The above data suggest that STX and neo-STX (Figure 1A,B) should be determined independently with specific antibodies coated to the solid phase as well as using specific toxin−HRP in each assay. Consequently, extensive efforts were made to optimize the ELISA conditions. The standard curves of the dc-ELISA of STX and neo-STX in these systems are shown in Figure 2. The ID_{50} values for STX and neo-STX for the binding

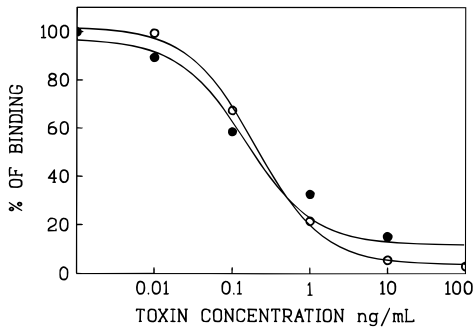

Figure 2. Standard curves of dc-ELISA of STX (○) and neo-STX (●). Specific polyclonal antibodies against either STX or neo-STX were coated to the wells of the ELISA plate as shown in Figure 1. STX−HRP and neo-STX−HRP conjugates were used for the analysis of STX and neo-STX, respectively. Data with no error bars indicate that the errors were within the size of the symbols.

of STX−HRP and neo-STX−HRP to the anti-STX and anti-neo-STX antibodies coated to the solid phase are found to be 0.28 and 0.18 ng/mL, respectively. The ID_{20} values for STX and neo-STX are found to be around 0.06 and 0.02 ng/mL, respectively.

Analytical Recovery of STX in a dc-ELISA. Results for the analytical recovery of STX added to the extracts of 4 different shellfish are given in Table 2. The overall recoveries, between 0.5 and 10 ng/mL (0.5−10 ppb), for STX in butter clams, Dungeness craba, Tanner crabs, and blue mussels were found to be 88.1 (CV 3.9%), 92.7 (CV 2.7%), 92.2 (CV 9.7), and 93.5% (CV 2.8%), respectively. Extract from the stomach contents of a geoduck was also tested; the recovery of STX added (0.5−10 ng/mL) was found to be 95% (CV 4.7%). The analytical recoveries for STX added to these extracts at 0.1 ng/mL all exceeded 100%; thus, these were excluded from the calculation of the overall average for the analytical recovery. These data also indicate that severe matrix interference occurred at the 0.1 ppb level in all the of matrices tested. Our data are consistent with the results of Usleber et al. (1991): analytical recovery of STX added to mussel and clam in a microtiter-based direct ELISA in the range of 10−1000 ng/g was found in the range of 75−88.5%. These investigators found that the detection limits for STX in mussel and clam were 3 and 4 ng/g, respectively.

Analysis of PSP Toxin Mixtures. Results for the analysis of three solutions that contain a mixture of three major groups of PSP toxins are given in Table 3. The toxin levels in each group were determined as STX and neo-STX with dc-ELISA. Results clearly show the high specificity of the ELISAs for both STX and neo-STX. Group TUMS-913 showed excellent agreement between the STX and neo-STX levels in the solution and the amount determined by ELISA. The levels of STX and neo-STX determined by ELISA were slightly higher than the actual level. These could be due to the presence of a small amount of decarbamoylated STX (DC-STX) in the solution because both anti-STX and anti-neo-STX cross-reacted weakly with the DC-STX. Due to the unavailability of the toxins, the cross-reactivity of both antibodies with gonyautoxins (GTXs) and group C toxins was not determined. However, data from Table 3 clearly show that both antibodies have good cross-reaction with GTX toxins. In particular, the presence of GTXs in the sample could readily be picked up by the dc-ELISA system when anti-STX antibodies were used. The total amount of PSP toxin, determined as STX in the mixture TUMG-913, was 3.83 μM, which

Table 2. Analytical Recovery of STX Added to the Shellfish Extracts

	butter clams		Dungeness crabs		Tanner crabs		blue mussels	
amt added[a] (ng/mL)	%	CV	%	CV	%	CV	%	CV
10	90.4	3.4	86.1	5.2	83.1	12.6	84.4	1.5
5	95.8	2.7	83.7	3.7	92.2	9.6	88.2	0.2
2	95.7	2.8	95.4	0.5	97.7	7.2	95.8	5.2
1	73.0	8.8	90.6	1.7	91.8	14.3	94.1	6.0
0.5	85.8	1.9	107.8	2.2	96.6	5.1	105.8	1.2
0.1	136.0	9.4	115.0	2.1	149.0	4.8	122.0	1.6
mean	88.1	3.9	92.7	2.7	92.2	9.7	93.5	2.8

[a] Each milliliter contains 1 g of tissue.

Table 3. Analysis of Solutions Containing a Mixture of PSP Toxins as STX or Neo-STX by ELISA

PSP toxin mixture	amt (μM)	data from dc-ELISA (μM) STX	neo-STX
TUMS-913			
STX	0.450	0.46	
neo-STX	0.977		1.18
Dec-STX	0.265		
TUMG-913	3.34[a]	3.83	1.14
TUOC-915	0.81[b]	ND[c]	ND

[a] Total concentration in this group, which contained GTX1, GTX2, GTX3, GTX4, and GTX5 at concentrations of 1.52, 0.444, 0.154, 0.520, and 0.698 μM, respectively. [b] Total concentration in this group, which contained C1, C2, C3, and C4 at concentrations of 0.555, 0.145, 0.0908, and 0.0205 μM, respectively. [c] ND, none detectable.

is close to the total amounts of GTXs (3.34 μM) in the mixture. The cross-reaction of some GTXs with anti-neo-STX is also apparent from data present in Table 3, but their reactivity was apparently less than with the anti-STX. These results are different from those reported in a previous study in which Usleber et al. (1991) found that GTX did not cross-react appreciably with the polyclonal antibodies against STX. In a later study, these investigators (Usleber et al., 1994) found that the cross-reactivity of the anti-STX antibodies with different STX analogoues varied with the markers used in the test. In the dc-ELISA using the anti-STX and STX–HRP system they found that GTX2/3 had about 10% of the cross-reactivity of as those for STX. In the present study, we found that both antibodies appear to have less reactivity with the C-type toxins. Neither STX nor neo-STX was detected in solution 3, which contained a total of 0.813 μM of the C toxins.

dc-ELISA of STX and Neo-STX in Naturally Contaminated Shellfish Samples. Results for analysis of STX and neo-STX in the viscera of Dungeness crabs, Tanner crabs, blue mussels, and oysters by dc-ELISA are presented in Figures 3 and 4. A linear regression for correlation of mouse assay data with the ELISA results for STX, neo-STX, and STX plus neo-STX in each group of shellfish was made. In addition, data within ±25% of the average of mouse assay results and total ELISA data (STX plus neo-STX) were selected for linear regression analysis. Detailed statistical results for Dungeness and Tanner crabs and for blue mussels and oysters are given in Tables 4 and 5, respectively. Several conclusions could be drawn from these data.

(*1*) A good linear correlation (coefficient of determination, r^2 values of 0.85 and 0.65 at $p < 0.0001$) between the total ELISA data and mouse assay results for the crab samples was found; the correlation improved (r^2 values of 0.89 at $p < 0.0001$) when one-third of the populations that had high mouse assay results were omitted from the calculations (Table 4).

(*2*) The linear correlation of the data obtained from

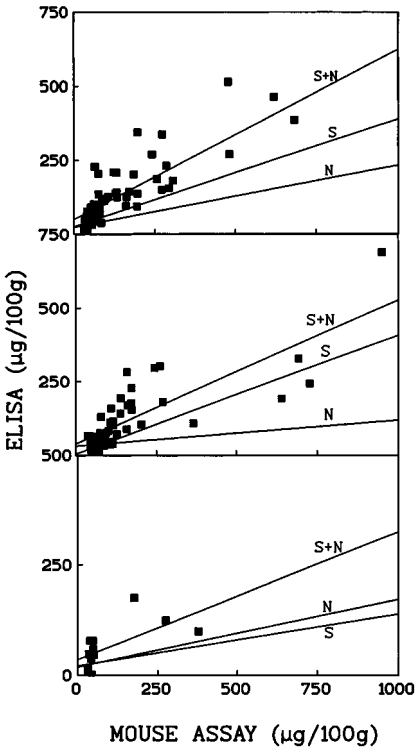

Figure 3. Correlation of STX, neo-STX, and STX plus neo-STX in 58 Dungeness crab (top), 47 Tanner crab (middle), and 13 oyster (bottom) samples as determined by dc-ELISA with mouse assay results. Individual data points for STX (S) and neo-STX (N) are omitted from the figures.

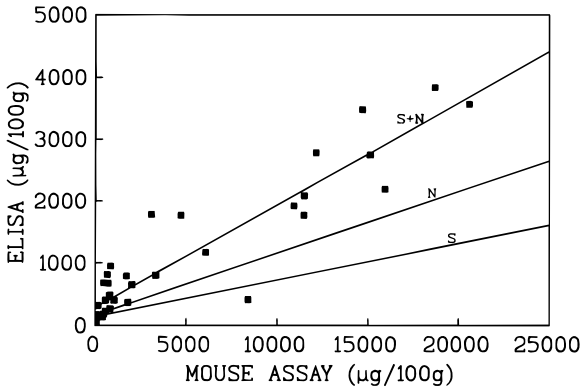

Figure 4. Correlation of STX, neo-STX, and STX plus neo-STX in 36 blue mussel samples as determined by dc-ELISA with mouse assay results. Individual data points for STX (S) and neo-STX (N) are omitted from the figures.

ELISA and mouse assay for the mussel and oyster samples (r^2 values of 0.86 and 0.43 at $p = 0.0001$ and 0.0135) was not as good as that for the crab samples. This was primarily because the ELISA values for several samples, especially mussel samples, were much less than those of mouse assays. Zinc concentrations

Table 4. Correlation of ELISA Data with Mouse Data for Dungeness Crab and Tanner Crab Samples[a]

shellfish analyzed:	Dungeness crabs			Tanner crabs		
PSP toxins:	sum[b]	neo-STX	STX	sum	neo-STX	STX
overall data						
no. analyzed	58	58	58	47	47	47
slope	0.57 (0.03)	0.21 (0.02)	0.37 (0.02)	0.49 (0.05)	0.09 (0.03)	0.40 (0.04)
ELISA at $M = 0$[c]	49.5 (10.4)	23.5 (7.1)	25.7 (6.1)	40.7 (13.7)	32.9 (8.9)	5.8 (9.9)
ELISA at $M = 80$[d]	95.1	40.3	62.7	79.9	40.1	37.8
r^2	0.85	0.61	0.86	0.65	0.12	0.71
P values	*	*	*	*	0.0166	*
selected data						
no. analyzed	38	38	38	31	31	31
% of total population	65.5			66		
slope	0.71 (0.04)	0.28 (0.04)	0.43 (0.03)	0.74 (0.05)	0.12 (0.03)	0.62 (0.04)
ELISA at $M = 0$	33.8 (10.6)	16.2 (8.8)	18.4 (6.9)	34.9 (11.0)	38.7 (11.9)	−6.6 (7.2)
ELISA at $M = 80$	90.6	38.6	55.2	94.2	40.3	43.0
r^2	0.89	0.63	0.87	0.88	0.14	0.92
P values	*	*	*	*	0.037	*

[a] Values in parentheses are SD. [b] Sum of neo-STX and STX. [c,d] Calculated ELISA data at mouse assay of 0 and 80 μg/100 g, respectively. * $p < 0.0001$.

Table 5. Correlation of ELISA Data with Mouse Data for Blue Mussel and Oyster Samples[a]

shellfish analyzed:	blue mussels			oyster		
PSP toxins	sum[b]	neo-STX	STX	sum	neo-STX	STX
overall data						
no. analyzed	36	36	36	13	13	13
slope	0.17 (0.01)	0.10 (0.01)	0.06 (0.005)	0.27 (0.09)	0.12 (0.06)	0.15 (0.05)
ELISA at $M = 0$[c]	283 (88.5)	165 (86.7)	140 (41.3)	40.9 (13.3)	21.6 (8.7)	19.4 (7.2)
ELISA at $M = 80$[d]	299.6	173	143	62.5	31.2	31.4
r^2	0.86	0.70	0.78	0.43	0.26	0.46
P values	*	*	*	0.0135	0.0745	0.0107
selected data						
no. analyzed	10	10	10	8	8	8
% of total population	28			61.5		
slope	0.93 (0.16)	0.50 (0.11)	0.36 (0.14)	0.95 (0.09)	0.60 (0.06)	0.34 (0.05)
ELISA at $M = 0$	33.1 (83.9)	−21.7 (59.6)	54.6 (74.7)	9.48 (13.3)	0.02 (8.7)	9.46 (7.2)
ELISA at $M = 80$	107.5	18.3	83.4	77.5	48	36.7
r^2	0.81	0.71	0.45	0.94	0.90	0.89
P values	0.0004	0.0022	0.034	*	0.0004	0.0005

[a] Values in parentheses are SD. [b] Sum of neo-STX and STX. [c,d] Calculated ELISA data at mouse assay of 0 and 80 μg/100 g, respectively. *, $p < 0.0001$.

in the oysters may also interfere with mouse bioassay. The slopes in the linear regressions were also low. However, the slopes increased considerably after these data were omitted from calculations. About 28 and 61.2% of the populations provided good correlation in the mussel and oyster samples.

(*3*) More STX was apparently present in the crab samples, and the correlation of STX with mouse assay results was better than that for neo-STX with mouse assay results. On the other hand, neo-STX appears to be a primary contributor in the blue mussel and oyster samples.

(*4*) Because mouse assays always gave higher values than ELISA data when the toxin levels were high and lower values than ELISA when the toxin levels were low, the slopes of all the linear regression analyses are lower than 1.0. These results are consistent with the inherent problems of mouse assays at very high and very low levels of toxin in the samples. Nevertheless, in considering the intercept values at the ELISA data axes, all of the ELISA data provided excellent prediction for the mouse data, with the exception of mussel samples. At the regulatory level of 80 μg of PSP/100 g of sample by mouse assay, the calculated ELISA data for dungeness crabs, tanner crabs, mussels, and oysters were 95.1, 79.9, 299.6, and 62.5, respectively. Thus, the ELISA method could be used for screening of PSP in crabs and osysters if a ± 20% experimental error is built into such consideration.

(*5*) Data from two-thirds of the mussel samples by mouse assay were considerably higher than those obtained from ELISA. Several factors could contribute to this problem: (*i*) the inaccuracy of mouse assay at high toxin levels; (*ii*) the instability of neo-STX in the samples; (*iii*) the presence of C groups of toxins in the samples.

Conclusion. In the present study, several dc-ELISA protocols have been established for the analysis of both STX and neo-STX. Although each protocol has its own merits for the determination of each of these toxins, we found that the dc-ELISA involving the use of specific antibodies against either STX or neo-STX together with specific toxin−HRP marker is most versatile. The assays provided both sensitivity and specificity for the major toxins involved in the PSP incidences. As low as 0.5 ppb of STX or neo-STX could be measured in the ELISA. In the naturally contaminated samples, low toxin levels (e.g. 3−10 ng/g) were detected in some samples. There was good correlation between the ELISA data and results obtained from the mouse assay performed independently in a separate laboratory. To cover most toxins involved in PSP in the naturally contaminated samples, two ELISAs should be conducted simultaneously. The overall data are also consistent with another study in which more than 700 samples of different matrices were subjected to an indirect ELISA (Chu, Hsu, and Huang, unpublished observation). The indirect competitive ELISA could identify the positives

(100%) shown by the mouse assay at the threshold level of 80 μg of toxin/100 g of sample. The extrapolated data from the present study indicate that the dc-ELISA may offer an alternative to bioassay if further modifications improve correlation with the standard screening bioassay. With these two assays used in the present study, ELISA may still miss some toxic samples if high levels of the C group toxins are present in the samples because large amounts of C group toxins have been reported in some incidences (Okumura et al., 1994). Thus, research should be directed to developing an ELISA method that could cover all of the PSP toxins and to develop specific antibodies that could detect the C group of toxins. Further studies are also needed to study the correlation of ELISA data with those from HPLC analysis.

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