Table II. Physical Properties of Dowco 417

physical state	light yellow oil
vapor pressure	1.54×10^{-6} mmHg at 26 °C[a]
solubility (water)	0.2 ppm at 26 °C[b]
1-octanol-water distribution coefficient	640 000[c]
average soil adsorption coefficient (K_{OC})	46 000[d]

[a] Determined by the effusion method (Hamaker and Kerlinger, 1969). [b] Determined by the nephelometric method (Davis and Parke, 1942). [c] Determined by the method of Fujita et al. (1964). [d] Based on soil-water slurry measurements in 20 soils with an organic carbon content of 0.0976-1.3%; determined by the method of Grover et al. (1979).

Table III. Toxic Properties of Dowco 417

acute oral LD_{50} (rat)	460 mg/kg[a]
acute oral LD_{50} (mouse)	100-200 mg/kg[a]
dermal LD_{50} (rabbit)	>625 mg/kg[a]
skin and eye (rabbit)	nonirritating[a]
TLM_{48} (goldfish)	4.8 ppb[b]

[a] Keeler and Lockwood (1978). [b] Kurihara and Larson (1980).

When the method of Ames (1971) was used, Dowco 417 was found to be nonmutagenic (Brusick, 1977). Some individuals with known "sensitive skin" may experience a skin reaction if exposed to Dowco 417. The phenomenon is common to synthetic pyrethroids (World Health Organization, 1979). Its high fish toxicity is not much different from that of commercial pyrethroids (Kurihara and Larson, 1980), and in light of extremely low effective dosage for crop pest control and its immobility in soil, Dowco 417 should present a minimal hazard to fish.

Conclusion. Dowco 417 belongs to a new and extremely important generation of insecticides which possesses low mammalian toxicity and offers a large number of other significant advantages over conventional insecticides. It is highly active against important foliar pests, especially sucking insects, and because of its lower application rates, plants do not show any foliar injury, thus resulting in higher crop yields. In view of its low use rates and relatively rapid breakdown in the environment, this material would involve little risk of environmental contamination.

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Specific Toxicity of Paralytic Shellfish Poisons

The specific toxicity of six paralytic shellfish toxins, neosaxitoxin and gonyautoxins-I, -II, -III, -IV, and -V, in mice was established in an equivocal manner, and their relative toxicity to saxitoxin was calculated. The method used is based on determinations of the nitrogen content of a toxin solution with a known number of mouse units and is not affected by isomerization, degradation, or the hygroscopic nature of the toxin. No clear structure–activity relationship was observed.

Paralytic shellfish poisoning, PSP (Figure 1), has been a serious problem for a long time in many parts of the world. The sporadic and unpredictable outbreaks usually cause serious health hazards and great losses to the seafood industry. The toxins accumulate in shellfish as a result of ingestion of toxic dinoflagellate. Saxitoxin (STX), first isolated from Alaska butter clams, *Saxidomus giganteus*, and later from California mussels, *Mytilus californianus* (Schantz et al., 1957), was thought to be the only toxic principle produced by the causative organism *Gonyaulax catenella* (Schantz et al., 1966). Recent studies, however, have shown that the toxicity is caused by a group of closely related compounds and that saxitoxin did not even constitute the major component in many cases (Shimizu et al., 1978a). Toxins of *Gonyaulax tamarensis*, the causative organism of PSP in the North Atlantic region, was also studied in our laboratory and was found to contain more than seven toxins, including saxitoxin (Shimizu et al., 1975; Oshima et al., 1977; Hsu et al., 1979).

The toxicity of saxitoxin was assigned to be 5500 mouse units (mu)/mg of the dihydrochloride or 1 mouse unit is equivalent to 0.18 μg of saxitoxin dihydrochloride (Schantz et al., 1958). Regarding other toxins, there is no description of their specific toxicity except for two toxins isolated from toxic scallops (Boyer et al., 1978b), but there is no complete list of specific toxicity of all the toxins determined under

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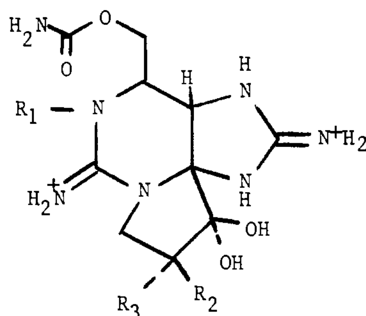
Table I. Specific and Relative Toxicity of PSP Toxins

	STX	neoSTX	GTX-I	GTX-II	GTX-III	GTX-IV	GTX-V
mouse units analyzed	1524 ± 94	1772 ± 76	598 ± 47	522 ± 55	1680 ± 103	392 ± 22	238 ± 13
N_2 found: μg (N_2)	72.94	168.96	35.56	64.50	73.68	57.02	66.56
μmol (toxin)	0.745	1.724	0.365	0.658	0.752	0.582	0.673[a]
mu/μmol	2045 ± 126	1038 ± 44	1638 ± 128	793 ± 83	2234 ± 137	673 ± 38	354 ± 19
sp toxicity, mu/mg	5494 ± 339[b]	2363 ± 101[c]	3976 ± 312[d]	2003 ± 211[d]	5641 ± 346[d]	1634 ± 92[d]	
rel toxicity	100	43	72	36	103	30	

[a] Based on the assumption that GTX_5 also contains seven nitrogen atoms. [b] As dihydrochloride. [c] As diacetate. [d] As monoacetate.



	R_1	R_2	R_3
Saxitoxin	H	H	H
Neosaxitoxin	OH	H	H
Gonyautoxin-I	OH	H	OSO_3^-
Gonyautoxin-II	H	H	OSO_3^-
Gonyautoxin-III	H	OSO_3^-	H
Gonyautoxin-IV	OH	OSO_3^-	H

Figure 1. Structures of paralytic shellfish poisons.

the same standardized conditions. Such specific toxicity data are essential in order to develop physicochemical assays of the PSP toxins. Also, interest in STX and other PSP toxins has been growing rapidly since these molecules proved to be of great value for studying the structure of the sodium channel or the excitable membrane (Narahashi, 1972). Associated with such interest was a need to explicitly determine the specific toxicity of individual toxins, a process which has been impeded by several factors which include (a) the unstability of the toxins and their ease of isomerization at high pHs (Shimizu, 1978), (b) the strong hygroscopic nature of the toxins which makes accurate weighing very awkward and unreliable, and (c) the difficulty in obtaining pure samples due to the minuteness of quantities and their close chromatographic behavior. It was, therefore, our objective to determine their specific toxicity with pure samples in a way not affected by the above-mentioned problems.

MATERIALS AND METHODS

Isolation of the Toxins. Toxins used in this study were isolated from the toxic sea scallop, *Placopecten magellanicus*, collected from the waters of the Bay of Fundy in Oct 1977 and from cultured *G. tamarensis*, Ipswich isolate, obtained from C. Martin of the Marine Laboratory, University of Massachusetts, Gloucester, MA. The organism was isolated during the massive outbreak in 1972 along the coastal areas of New England and has been maintained in our laboratory since then. The standard sample of saxitoxin used in this study was obtained through the U.S. Food and Drug Administration; its homogeneity was checked by thin-layer chromatography and was used to standardize the mice used for bioassay and also for calibration of the C and N analyzer. The extraction and purification of the toxins were carried out either from the toxic scallop from the Bay of Fundy or from cultured *G. tamarensis* cells. In the latter case the culture was passed through a column packed with glass wool where the cells are adsorbed to the glass wool fibers. This is followed by extraction with acidified (pH 2.3) aqueous ethanol. In either case the extract was purified following the previously reported method (Oshima et al., 1977; Hsu et al., 1979). The separation of individual toxins was carried out by ion-exchange chromatography using a Bio-Rex 70 resin column (H^+ form; –400 mesh; 1.7 × 90 cm; Bio-Rad Laboratories, Rockville Center, NY) and an acetic acid gradient mixture for elution (Hsu et al., 1979). The purity of individual toxins was confirmed by thin-layer chromatography on Whatman high-performance LHP-K plates using the solvent system pyridine–ethyl acetate–water–acetic acid (75:50:20:15). Fractions showing a single pure toxin were then lyophilized and dissolved in a known volume of glass-distilled water.

Mouse assay of the isolated pure toxins was carried out immediately after confirming their purity. The bioassay was carried out by using 20–22-g male mice (outbred Albino; CD-1; Charles River Breeding Laboratories, Wilmington, MA) following the established AOAC method (Schantz et al., 1958). Ten mice were used for each sample in order to determine the number of mouse units per unit volume of the toxin solution.

Preparation of Toxin Samples for Analysis. Aliquots of the toxin solutions containing known mouse units (Table I) were transferred to combustion boats which had been incinerated at 400 °C for 18 h prior to their use. The toxin solutions were dried in an oven at 85 °C and kept in a desiccator until ready for analysis. Four boats were prepared for each toxin. Concurrently, similar treatment was done on (a) glass-distilled water, (b) toxin-free fractions from the Bio-Rex 70 column, and (c) a solution of standard saxitoxin which has been mouse assayed to be used as a reference standard for calibration.

The C and N analyzer (Hewlett-Packard HP-185 B carbon, nitrogen, and hydrogen analyzer) was checked before running the samples by using blank boats treated with glass-distilled water and then calibrated with a standard solution of saxitoxin dihydrochloride. Saxitoxin was chosen as a standard because it was expected to afford the closest nitrogen recovery ratio due to its structural similarity to other toxins. From the results obtained with saxitoxin, the analyzer integrator was calibrated.

RESULTS AND DISCUSSION

Purity of the Bio-Rex 70 column fractions was always checked by TLC, and when pure fractions were obtained, they were combined, lyophilized, and immediately bioas-

sayed by using mice. Purity of the samples was checked again after the mouse assay in order to ascertain that the bioassay was carried out on a homogeneous sample. Isomerization of the toxins after the mouse assay would have no effect on the analytical results. Thus the analysis of nitrogen or carbon contents of a known number of mouse units of the toxin solutions should enable us to calculate the amount of the toxins analyzed and subsequently their accurate specific toxicities. This approach certainly precludes the difficulties associated with weighing of minute quantities of the hygroscopic toxins and should not be affected by either isomerization of degradation during handling. In this study nitrogen analysis data were used instead of those of carbon because of the heterogenecity of the counterions. The counterions of individual toxins might be anticipated as acetate ions. However, since we are handling very minute amounts of toxins, the counterions of the lyophilized samples were actually a mixture of acetate and other inorganic anions such as chloride ions left on the column in trace amounts as was reflected by the fluctuation in the carbon contents.

The reliability of the method was checked by two methods. (1) The first was to treat toxin-free fractions from the Bio-Rex 70 column in a similar manner to eliminate the possibility of nontoxin nitrogeneous compounds coming out of the column that might alter the results. All such fractions gave zero readings for N_2. (2) In the second method, saxitoxin, isolated from scallop and treated in the same way as the other toxins, gave a specific toxicity of 5494 ± 339 mu/mg (by the new procedure) as compared to the 5500 mu/mg value reported earlier and checked in our laboratory by the standard mouse assay procedure.

The number of mouse units analyzed of each toxin and the data obtained from the nitrogen analysis are listed in Table I. The number of mouse units per milligram of each toxin was calculated on the basis of the known structures of the toxins except in the case of GTX-V whose exact structure is still being studied in our laboratory. It is, however, assumed that GTX-V has the same ring skeleton as in other toxins and, hence, contains seven nitrogen atoms in the molecule, and the number of mouse units per micromole was calculated based on such assumption. The results of N_2 analyses of the quadruplets of each toxin were in excellent agreement, and the average of the four values was used for calculation of the specific toxicity of neoSTX and GTX-I to -IV.

The structure of neosaxitoxin was reported as 1-hydroxysaxitoxin (Shimizu et al., 1978b) while the structures of GTX-II and GTX-III were first reported by Shimizu as 11α-hydroxysaxitoxin and 11β-hydroxysaxitoxin (Shimizu et al., 1976) and was later revised by Boyer as the sulfate esters rather than the hydroxy derivative (Boyer et al., 1978a). The structures of GTX-I and GTX-IV have also been recently proposed as 11α-hydroxyneosaxitoxin sulfate and 11β-hydroxyneosaxitoxin sulfate, respectively (Shimizu, 1979).

The results obtained indicate that the substitution at N-1 and/or C-11 shows a tendency to decrease the toxicity except in the case of GTX-III which showed toxicity comparable to that of STX, yet all compounds retain the same order ot toxicity. From the data obtained, no plausible correlation between the structures and the toxicity could be deduced.

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