Brevetoxin Forms Covalent DNA Adducts in Rat Lung Following Intratracheal Exposure

Faisal F.Y. Radwan and John S. Ramsdell

Background: Human exposure to brevetoxins produced by the red tide organism, Karenia brevis, is an increasing public health concern. Using in vitro exposure of rat liver cells to brevetoxin B (PbTx-2), the primary toxin product of K. brevis, we previously showed that it formed C27,28-epoxy brevetoxin metabolites capable of covalently binding to nucleic acids, a common initiation step for carcinogenesis.

Objective: This study was undertaken to evaluate nucleic acid adduction in lung following in vitro and in vivo brevetoxin exposures.

Methods: To clarify reactions of brevetoxin epoxide with DNA, we analyzed reaction products of PbTx-6 (a C27,28 epoxide metabolite of brevetoxin B) with nucleosides. We also analyzed adducts from nucleic acid hydrolysates of isolated rat lung cells treated with PbTx-2 or PbTx-6 in vitro and lung tissue from rats after intratracheal exposure to PbTx-2 or PbTx-6 at 45 μg toxin/kg body weight.

Results: Our results indicate that PbTx-2 forms DNA adducts with cytidine after treatment of isolated lung cells, and forms DNA adducts with adenosine and guanosine after intratracheal exposure. Conclusions: These results are consistent with metabolic activation of highly reactive brevetoxin intermediates that bind to nucleic acid. These findings provide a basis for monitoring exposure and assessing the hazard associated with depuration of brevetoxin–nucleotide adducts in lung tissue.

Key Words: brevetoxin, DNA adducts, epoxidation, harmful algal bloom, Karenia brevis, lung, metabolism.

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phase II enzymes such as epoxide hydrolase, UGT1A (glucuronyl transferase), and GST-P1 (glutathione S-transferase), which largely act as detoxifying enzymes.

Adduct formation is a key step in inducing genotoxicity as a result of exposure to chemicals and metabolites that damage nucleic acid. The present study aimed to investigate brevetoxin adduction with nucleic materials in the lungs of exposed rats and thus to provide insight into DNA adduction by brevetoxins following metabolic activation. Detection of brevetoxin–nucleic acid adduction has pharmacological and risk assessment importance and should provide a foundation for biological monitoring of human exposure to such genotoxic environmental agents.

Materials and Methods

Materials. Brevetoxins PbTx-2 (MH+, m/z 895) and PbTx-6 (MH+, m/z 911), derived from K. brevis cultures [purity reported by vendor to be ≥ 95% by high performance liquid chromatography (HPLC)], were purchased from EMD Chemicals, Inc. (San Diego, CA, USA). Nucleosides guanosine and cytosine were purchased from Calbiochem (EMD Biosciences, Inc., San Diego, CA, USA). Total nucleic acid was isolated using DNeasy Tissue Kit produced by Qiagen Inc. (Germantown, MD, USA). Dulbecco’s modified Eagle medium (DMEM) was purchased from Invitrogen (Carlsbad, CA, USA). Rat lung cells (CCL-192/RFL-6) were purchased from the American Type Culture Collection (Manassas, VA, USA). All other analytical or molecular biologic grade chemicals used in this study were purchased from either Sigma Chemical Co. (St. Louis, MO, USA) or Fisher Scientific (Suwanee, GA, USA).

Semisynthesis of nucleoside adducts with PbTx-6. Reaction mixtures (200 µL total volume) of PbTx-6 (200 µM) were incubated with aliquots of 50 mM equivalents of individual nucleosides prepared in 100 mM phosphate buffer at pH 7.4. The incubation was carried out in a shaking bath overnight at 37°C. The reaction mixture was centrifuged at 900 × g for 5 min to remove any particulates. We isolated the brevetoxin nucleoside adducts in the supernatant using a 500 mg C-18 cartridge (Varian; Palo Alto, CA, USA) previously conditioned with 3 mL methanol followed by 3 mL distilled water. Reactions were loaded, washed with 3 mL 10% methanol, and adducts were eluted with 5 mL 85% methanol. We dried and reconstituted adducts in 50% methanol for further radioimmunoassay (RIA) analysis. We initiated toxin exposure by adding PbTx-2- or PbTx-6-enriched medium to 2 a well containing cells. The final incubation mixture contained 35 µM of toxin and a cell count of approximately 1.1 × 10^6 cells/mL. Control reactions were performed by incubating cells in brevetoxin-free medium. We determined cell viability (> 86%) before exposure using the trypan blue (0.4%) exclusion test. All incubations were performed at 37°C for 18 hr in a 5% CO₂ humidified atmosphere. Cells were pelleted by centrifugation at 1,500 × g for 10 min and immediately frozen at −80°C for further nucleic acid extraction.

Rat intratracheal exposures to PbTx-2 and PbTx-6. Brevetoxin treatments were carried out at Charles River Laboratories Inc., (Horsham, PA, USA) using 12-week-old Charles River male rats [Crl:CD(SD)IGS BR VAF/Plus; 225–250 g body weight]. Animals were separated into three groups of five rats per group and identified using Monel self-piercing ear tags. Brevetoxins dissolved in 10% methanol were further diluted in physiological saline containing 0.01% Emulphor EL-620 (GAF Corp., New York, NY, USA). Rats in the treatment groups were administered PbTx-2 (group 1) or PbTx-6 (group 2) at 45 µg/kg body weight through intratracheal instillation. We determined the dosage by a preliminary range-finding study of the maximally tolerable dose for PbTx-2 as defined by labored breathing not greater than 30 min after exposure. Briefly, rats were anesthetized using 5% isoflurane in O₂ and intubated intratracheally as described previously (Medinsky et al. 1986). The doses, in a volume of 0.15 mL saline, were instantaneously delivered directly to the lung by a Luer-Lok syringe through a 16-gauge catheter. Rats were killed 24 hr after treatment, and the lungs were collected immediately and kept frozen at −80°C until use. Control rats received brevetoxin-free vehicle solution (group 3). Animal use was conducted according to Good Laboratory Practice Procedures, and the animals were treated humanely and with regard for alleviation of suffering. The experimental protocol was approved by the Charles River Laboratories Institutional Animal Care and Use Committee.

Nucleic acid extraction and digestion. We isolated nucleic acid from rat lung using Qiagen Genomic-tips, essentially as described by the manufacturer, with the following modifications. Cultured cells (~ 3.3 × 10^9/test) were thawed and resuspended in 200 µL phosphate buffer (0.1 M). Lung tissues were minced into smaller pieces in a Petri dish containing Dulbecco’s phosphate-buffered saline and preincubated the preparation at 37°C for 10 min. Aliquots of rat lung cell culture suspension were transferred into new incubation wells and preincubated at 37°C for 10 min. We initiated toxin exposure by adding PbTx-2- or PbTx-6-enriched medium to a well containing cells. The final incubation mixture contained 35 µM of toxin and a cell count of approximately 1.1 × 10^6 cells/mL. Control reactions were performed by incubating cells in brevetoxin-free medium. We determined cell viability (> 86%) before exposure using the trypan blue (0.4%) exclusion test. All incubations were performed at 37°C for 18 hr in a 5% CO₂ humidified atmosphere. Cells were pelleted by centrifugation at 1,500 × g for 10 min and immediately frozen at −80°C for further nucleic acid extraction.

In vitro treatment of lung cells with PbTx-2 and PbTx-6. We prepared toxins in methanol and diluted them in the culture medium (DMEM) so that the final methanol concentration was 1.5% (vol/vol); we then

**Figure 1.** Schematic diagram showing the intermediary metabolites generated after in vitro metabolic bioactivation pathways of brevetoxin-B in rat liver cells.
brevetoxin–cytidine (B), brevetoxin–guanosine (C), and unbound PbTx-6 (D). The brevetoxin-adduct fragment patterns shown in (B) and (C) depict a hypothetical LC/MS analysis of semisynthesized brevetoxin nucleic acid adducts. Rel Int, relative intensity. (A) Extracted ion current (m/z 860–1,400) from total ion Figure 2.

Figure 3. LC/MS analysis of semisynthesized brevetoxin nucleic acid adducts. Rel Int, relative intensity. (A) Extracted ion current (m/z 860–1,400) from total ion chromatograms for brevetoxin–cytidine, brevetoxin–guanosine, and unbound PbTx-6. (B–D) Extracted total ion chromatogram, mass spectra, and product for brevetoxin–cytidine (B), brevetoxin–guanosine (C), and unbound PbTx-6 (D). The brevetoxin-adduct fragment patterns shown in (B) and (C) depict a hypothetical nucleoside linkage to the epoxide to illustrate potential nucleoside fragmentation.

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Brevetoxin–nucleic acid adduction in rat lung

50% methanol for immediate RIA or liquid chromatography (LC)/MS analyses.

RIA. We performed an RIA selective for toxins with the B-type backbone brevetoxin as described previously (Woof et al. 2003). Tests were carried out in borosilicate glass tubes using antibodies raised in sheep immunized with PbTx-2–fetuin conjugate (Garthwaite et al. 2001). Assays were conducted in RIA buffer, a PBS containing 0.01% Emulphor-EL 620 with a final volume of 500 µL/test. Brieﬂy, 5 µL of adduct extract, or PbTx-3 (the PbTx-2 terminal alcohol reduction product) standard set (from 0.01 to 1,000 nM) was incubated with 20 µL anti-PbTx antiserum (1:4,000) in RIA buffer for 1 hr at 25°C. Thereafter, 0.4 nM of the \(^{3}\)H-PbTx-3 tracer was added, and the incubation proceeded for another hour. Finally, we added 100 µL Sac-Cel (IDS Diagnostics, Fountain Hills, AZ, USA) to each of the assay tubes allowing the separation of the bound and unbound brevetoxin. The Sac-Cel mixture was ﬁltered onto 25-mm diameter GF/B glass ﬁber ﬁlters (Whatman, New York, MA, USA). The ﬁlters were immersed in 5 mL Scinti-verse (Fisher, Suwannee, GA, USA) for 18 hr, and the radioactivity was counted on a Packard Bioscience Company, Meriden, CT, USA). The filters were immersed in 5 mL PBS containing 0.1% formic acid as an assay limit of detection for interference. The assay limit of detection for PbTx-3 equivalents per microgram DNA for cells exposed to PbTx-6 (m/z 1,154) was retained at about 18.5 min, identical for both control and treatments (Figure 3C). Fragmentation of nucleoside adducts resulted in initial elimination of the ribose moiety from the parent compounds: m/z 124 and m/z 152 for cytosine or guanine, respectively. Fragments of nucleic acid imine were seen as m/z 124 (C=N\(^{-}\)) and m/z 164 (C=N\(^{2}\)) for cytosine or guanine, respectively. PbTx-6 was characterized by its daughter fragments m/z 857 and m/z 473 (Figure 3C).

Adduct formation after in vitro exposure. Lung cells exposed to PbTx-2 showed an approximate 4-fold increase in the amount of adduct compared to PbTx-6 treatment. RIA showed 115.5 ± 31 pg PbTx-6 equivalents per microgram DNA for cells exposed to PbTx-2 and 34.1 ± 3 pg/tg DNA of cells exposed to PbTx-6. LC/MS analysis of extracts of PbTx-2 exposure revealed molecular ions corresponding to products between brevetoxin and cytidine. These ions included m/z 1,022 (brevetoxin–nucleobase after losing the sugar moiety) and cytosine m/z 112 and cytosine N\(^{4}\) imine m/z 124 (Figure 4). Neither adduct parent molecule nor its legitimate sugar-free ion fragment was detected in PbTx-6 exposures.

Adduct formation after in vivo exposure. A considerable amount of brevetoxin activity was retained in lung tissue 24 hr after exposure. An average of 136.3 ng PbTx-3 equivalents/g of lung tissue was measured for PbTx-2, while almost 2-fold more was measured in PbTx-6–exposed rats. RIA analyses of the nucleic acid hydrolysate showed about 4-fold greater adduct formation caused by PbTx-2 exposure (Figure 5). Further LC/MS analysis of the

**Figure 4.** LC/MS of brevetoxin–nucleic acid adducts formed by reaction of PbTx-2 with isolated rat lung cells shown as extracted ion current (m/z 860–1,400) from (A) a total ion chromatogram, (B) mass spectra, and (C) product ion spectra. Rel Int, relative intensity. The spectra shows brevetoxin–cytidine adduct molecule (m/z 1,154) and products (m/z 1,022, m/z 124, and m/z 112) resulting from fragmentation of the nucleoside. The brevetoxin adduct fragment pattern shown depicts a hypothetical nucleoside linkage to the epoxide to illustrate potential nucleoside fragmentation.

**Figure 5.** RIA of brevetoxin activity retained in lung tissues or associated with DNA extracts 24 hr after in vivo exposures to PbTx-2 or PbTx-6. Data shown are mean ± SD of PbTx-3 equivalents.
nucleic acid hydroxylate of PbTx-2–exposed lung revealed three molecular ions corresponding to brevetoxin adducts with nucleosides. The brevetoxin–guanosine adduct retained around 16 min (m/z 1,194) (Figure 6A), brevetoxin–adenine (m/z 1,046) retained at about 14 min (Figure 6B), and the brevetoxin–adenosine adduct retained at about 10.7 min (m/z 1,178; Figure 6C). Fragmentation spectra of nucleosidic adducts showed initial elimination of the sugar moiety to give daughter compounds: m/z 1,194 to m/z 1,062 for brevetoxin–guanosine and m/z 1,178 to m/z 1,042 for brevetoxin–guanosine. The loss of respective nucleobase imine was monitored in both adducts giving rise to ions m/z 152 or m/z 136 for guanine or adenine, respectively. Fragments of nucleobase imine were seen as m/z 164 (C=N2–) and m/z 148 (C=N3–). We could not detect adduct formation in hydrolysate extracts from PbTx-6 exposures using similar LC/MS conditions.

**Discussion**

Brevetoxin, the red tide toxin, is well characterized for its neurotoxic effects mediated by the voltage-gated sodium channel that are manifest in NSP (Ramsdell 2008). Respiratory exposure to seawater aerosols containing brevetoxins leads to a different constellation of effects (Bachelor et al. 2005), some of which are consistent with activation of voltage-gated sodium channels, whereas others may involve inflammatory reaction pathways (Bossart et al. 1998). Brevetoxins can also lead to potential genotoxic effects. *In vitro* studies with human lymphocytes indicated that brevetoxins cause single-stranded and and double-stranded DNA breaks (Sayer et al. 2005), and a follow-up study in Chinese hamster ovary cells reported that brevetoxin causes chromosomal aberrations (Sayer et al. 2006).

Systemic absorption of brevetoxins by mammals leads to rapid metabolism to produce more readily excreted polar products (Radwan et al. 2005). The initial, or phase I reactions of brevetoxin include oxidation, reduction, hydrolysis, and epoxidation (Radwan and Ramsdell 2006). Epoxidation of the H-ring of brevetoxin B leads to a series of reactive intermediates that are capable of covalently binding with DNA or causing oxidative DNA damage. Formation of reactive intermediates is an initial step in the carcinogenic mechanisms of other well-known natural toxins such as aflatoxin (Groupman et al. 2005). In the present study we examined the subsequent step in this progression—namely, the formation of brevetoxin–nucleoside adducts in lung after intratracheal exposure to brevetoxins. Adduct formation is a key step in inducing genotoxicity as a result of exposure to nucleic acid-damaging chemicals and metabolites.

**Mechanism for brevetoxin–DNA adduct formation.** We focused on brevetoxin B.

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**Figure 6.** LC/MS of brevetoxin–nucleic acid adducts formed by brevetoxin reaction in rat lung after intratracheal exposure. Rel Int, relative intensity. (A–C) Extracted ion current (m/z 880–1,400) from total ion chromatograms, mass spectra, and product ion spectra for (A) brevetoxin–guanosine m/z 1,194, (B) brevetoxin–adenine m/z 1046, and (C) brevetoxin–adenosine m/z 1,178, respectively. The brevetoxin adduct fragment patterns shown in the spectra of (A–C) depict a hypothetical nucleoside linkage to the epoxide to illustrate potential nucleoside or nucleotide fragmentation.
analogy; however, similar processes can be expected for brevetoxin A analogs. The initial brevetoxin B produced by the algae has a reactive \(\alpha,\beta\)-unsaturated aldehyde extending from the terminal K-ring and has been designated as PbTx-2. PbTx-2 is also subject to epoxidation, in the 27, 28 position of the H-ring designated PbTx-6, which as been found both in algal cells (Chou et al. 1985) and as a metabolic product of PbTx-2 in liver cells (Radwan and Ramsdell 2006). In this article, we first described the semisynthesis of brevetoxin–nucleic acid adducts produced from reactions of PbTx-6 with cytosine and guanosine. Reaction of PbTx-6 with nucleosides was noted by a decrease in toxin activity in the assay system compared to the control, which suggests that nucleosidic adducts may have a lower affinity for brevetoxin antibody. LC/MS data provided structural evidence for the formation of cova-
lent brevetoxin–cytidine and brevetoxin–guanosine adducts. The MS/MS spectrum of each adduct was identified by a parent \([M + PbTx-6+H]^+\) ion, and two major fragment ions: \([M + PbTx-6 – 132+H]\), probably formed by elimination of sugar moiety, and \([M + PbTx-6 – 132 – 18+H]\), likely due to further loss of water. Elimination of respective nucleobase was observed as \(m/z\) 112 and \(m/z\) 152 for brevetoxin–cytidine and brevetoxin–guanosine, respectively. Respective nucleobase imine fragment \(m/z\) 124 and \(m/z\) 164 were also observed, indicating that addition sites of nucleobases toward brevetoxin occurs at least in part on the exocyclic NH\(_2\) groups of the base moieties. LC/MS analysis did not yield sufficient structural information to confirm the site of nucleo-
side linkage to the H-ring epoxide.

**Characterization of brevetoxin–nucleoside adducts.** Brevetoxin–nucleoside adducts were examined after *in vitro* exposure of brevetoxins to lung fibroblasts and after *in vivo* exposure of rats to brevetoxins via intratracheal administration. Two brevetoxin congeners were chosen for exposure, PbTx-6, which has the H-ring epoxide, and PbTx-2, which has been found to be metabolized to form the H-ring epoxide in liver cells. Both brevetoxin congeners yielded brevetoxin immunoreactiv-
ity to purified nucleoside fractions recovered from exposed lung cells and lung tissue; how-
ever, in each case PbTx-2 yielded four times the activity found with PbTx-6 treatment. Extracted ion chromatograms identified breve-
toxin adducts only with PbTx-2 treatment. Brevetoxin–cytidine adducts were detected in PbTx-2–exposed lung cells, but no adducts were found in the nucleic acid extracts of lung cells exposed to PbTx-6 under the same LC/MS conditions. Adducts of brevetoxin–
guanosine \((m/z\) 1,194) and brevetoxin–adenosine \((m/z\) 1,178) as major products of hydrolyzed lung DNA after intratracheal exposure of rats. The finding of brevetoxin conjugated to guanosine and adenosine after hydrolysis of lung DNA is consistent with brevetoxin metabolism resulting in a reactive intermediate capable of reaching the nucleus from its site of metabolism in the mito-
chondria to react with DNA purines. DNA purine adducts are known to be unstable and to undergo depurination. The release of the nucleotide base adduct is an indication of a promutagenic lesion. Structurally, the lesion in the DNA is the result of an apurinic site that remains in the DNA base sequence. The identification of brevetoxin–nucleotide base (adenine) adduct \((m/z\) 1,046) in addition to brevetoxin–nucleoside (adenosine) adduct \((m/z\) 1,178) in rat lung tissue is consistent with the formation of promutagenic lesions after brevetoxin exposure. Promutagenic lesions resulting from depurination of afla-
toxin–nucleic acid adducts are susceptible to faulty repair, causing G to T transversions, which have been shown to result in inactiva-
tion of the TP53 tumor-suppressor gene in aflatoxin-associated hepatocarcinoma (Kensler et al. 2003).

**Potential risk of exposure to brevetoxin aerosols.** The risk of brevetoxin adduct forma-
tion and resultant depurination is not known. Individuals are continually exposed to envi-
ronmental agents capable of direct adduct for-
mation as well as adduct formation through environmental agents that induce oxidative damage. It is possible that exposure to breve-
toxins in aerosols, like other inhaled environ-
mental genotoxic carcinogens, including polyacromatic hydrocarbons (Schoket 1999) and tobacco smoke (Wiencke 2002), add to the baseline levels of DNA adducts. Baseline levels of adducts in humans are estimated to be in the range of 0.1–1.0 adduct per 10\(^8\) unmodified DNA bases, and this burden can rise by four orders of magnitude in animals exposed to carcinogenic levels of genotoxic agents (Singh and Farmer 2006). A question that remains is whether inhalation exposure to brevetoxin aerosols leads to significant lung DNA depurination. Key to this question is the rate of brevetoxin addition to DNA bases in lung and the extent of exposure.

The identification of brevetoxin–purine adducts now provides a foundation for biologi-
cal monitoring of human exposure to such genotoxic environmental agents. The dose lev-
els used in these *in vitro* and *in vivo* experi-
ments were not meant to be reflective of human inhalation exposure to brevetoxins, which has been estimated at 3 and 20 ng/hr for red tide events in Florida (2003) and Texas (2000), respectively (Cheng et al. 2005; Pierce et al. 2005). Rather, the dosage was high, by several orders of magnitude, to optimize the search for toxin–nucleoside adducts. Dose-
response studies are an important next step, and previous studies have shown that DNA adducts are formed on a linear basis to dose (Buss et al. 1990). However, most biological monitoring studies use readily obtained
samples, such as white blood cells for DNA adducts, serum for albumin adducts or urine for nucleotide adducts. The release of the nucleotide adduct from target tissue DNA as a result of depurination leads to the adduct elimination in urine. Identification of urinary nucleotide adducts using LC/MS has provided a foundation for large-scale monitoring studies of several genotoxic agents (Shuker and Farmer 1992; Singh and Farmer 2006) and could prove useful for brevetoxins as well.

**References**

Abraham WM, Bourdelais AJ, Sabater JR, Ahmed A, Lee TA, Serebrjakov I, et al. 2005. Airway responses to aerosolized brevetoxins during Florida red tide events. Harmful Algae 21(1):19–28.

Backer LC, Fleming LE, Rowan A, Cheng Y-S, Benson J, Pierce RH, et al. 2003. Recreational exposure to aerosolized brevetoxins in an animal model of asthma. Am J Respir Crit Care Med 171(1):28–34.

Fleming LE, Kirkpatrick B, Backer LC, Bean JA, Wanner A, Reich A, et al. 2007. Aerosolized red-tide toxins (brevetoxins) and asthma. Chest 131(1):187–194.

Garthwaite I, Ross KM, Miles CD, Briggs LR, Towers NR, Borrell T, et al. 2001. Integrated enzyme-linked immuno-sorbent assay screening system for amnesic, neurotoxic, diarrhetic, and paralytic shellfish poisoning toxins found in New Zealand. J AOAC Int 84(5):1643–1648.

Groopman JD, Johnsen D, Kessler TW. 2005. Aflatoxin and hepatitis B virus biomarkers: a paradigm for complex environmental exposures and cancer risk. Cancer Biomark 1(1):5–14.

Hertog PJ, Lindsay-Smith JR, Garner RC. 1980. A high pressure liquid chromatography study on the removal of DNA-bound aflatoxin B1 in rat liver and in vitro. Carcinogenesis 1:767–793.

Huang JM, Wu CH, Baden DG. 1984. Depolarizing action of a red-tide dinoflagellate brevetoxin on axonal membranes. J Pharmacol Exp Ther 229(2):615–621.

Kessler TW, Gian GS, Chen JG, Groopman JD. 2003. Translational strategies for cancer prevention in liver. Nat Rev Cancer 3(5):321–329.

Kirkpatrick B, Fleming LE, Backer LC, Bean JA, Tamer R, Kirkpatrick G, et al. 2006. Environmental exposures to Florida red tides: effects on emergency room respiratory diagnoses admissions. Harmful Algae 5(5):526–533.

Koskinen M, Pinta K. 2000. Specific DNA adducts induced by some mono-substituted epoxides in vitro and in vivo. Chem Biol Interact 129(3):209–226.

Lip Y-Y, Risk M, Ray SM, Van Engen D, Clardy J, Golik J, et al. 1981. Isolation and structure of brevetoxin B from the “red tide” dinoflagellate Phycodyschus brevis (Gymnodinium breve). J Am Chem Soc 103:9733–9737.

McFarren EF, Tanabe H, Silva FJ, Wilson WB, Campbell JE, Wiencke JK. 2002. DNA adduct burden and tobacco carcinogenesis. Proc Natl Acad Sci USA 99(24):15634–15639.

Mol Pharmacol 192(3):345–348.

Cheng YS, Villareal TA, Zhou Y, Gao J, Pierce RH, Wetzel D, et al. 2005. Characterization of red tide aerosol on the Texas coast. Harmful Algae 4(1):87–94.

Chou H-N, Shimizu Y, Van Duyne G, Clardy J. 1985. Isolation and structures of two new polycyclic ethers from Gymnodinium breve Davis (Phychodyschus brevis). Tetrahedron Lett 26(24):2865.

Davis C. 1948. Gymnodinium breve: a cause of discolored water and animal mortality in the Gulf of Mexico. Bot Gaz 109:356–360.

Fleming LE, Kirkpatrick B, Backer LC, Bean JA, Wanner A, Reich A, et al. 2007. Aerosolized red-tide toxins (brevetoxins) and asthma. Chest 131(1):187–194.

Garthwaite I, Ross KM, Miles CD, Briggs LR, Towers NR, Borrell T, et al. 2001. Integrated enzyme-linked immuno-sorbent assay screening system for amnesic, neurotoxic, diarrhetic, and paralytic shellfish poisoning toxins found in New Zealand. J AOAC Int 84(5):1643–1648.

Groopman JD, Johnsen D, Kessler TW. 2005. Aflatoxin and hepatitis B virus biomarkers: a paradigm for complex environmental exposures and cancer risk. Cancer Biomark 1(1):5–14.

Hertog PJ, Lindsay-Smith JR, Garner RC. 1980. A high pressure liquid chromatography study on the removal of DNA-bound aflatoxin B1 in rat liver and in vitro. Carcinogenesis 1:767–793.

Huang JM, Wu CH, Baden DG. 1984. Depolarizing action of a red-tide dinoflagellate brevetoxin on axonal membranes. J Pharmacol Exp Ther 229(2):615–621.

Kessler TW, Gian GS, Chen JG, Groopman JD. 2003. Translational strategies for cancer prevention in liver. Nat Rev Cancer 3(5):321–329.

Kirkpatrick B, Fleming LE, Backer LC, Bean JA, Tamer R, Kirkpatrick G, et al. 2006. Environmental exposures to Florida red tides: effects on emergency room respiratory diagnoses admissions. Harmful Algae 5(5):526–533.

Koskinen M, Pinta K. 2000. Specific DNA adducts induced by some mono-substituted epoxides in vitro and in vivo. Chem Biol Interact 129(3):209–226.

Lin Y-Y, Risk M, Ray SM, Van Engen D, Clardy J, Golik J, et al. 1981. Isolation and structure of brevetoxin B from the “red tide” dinoflagellate Phycodyschus brevis (Gymnodinium breve). J Am Chem Soc 103:9733–9737.

McFarren EF, Tanabe H, Silva FJ, Wilson WB, Campbell JE, Wiencke JK. 2002. DNA adduct burden and tobacco carcinogenesis. Proc Natl Acad Sci USA 99(24):15634–15639.

Mol Pharmacol 192(3):345–348.

Cheng YS, Villareal TA, Zhou Y, Gao J, Pierce RH, Wetzel D, et al. 2005. Characterization of red tide aerosol on the Texas coast. Harmful Algae 4(1):87–94.